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A Negro Family Revealing Hunter-Henshaw Information, and Independence of the Genes for Js and Lewis

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THE HUNTER (Landsteiner, Strutton and Chase, 1934) and Henshaw (Ikin and Mourant, 1951) human erythrocytic antigens are associated with the MNS blood group system, and both antigens occur almost exclusively in Negroes. Chalmers, Ikin, and Mourant (1953) found Hu (Hunter) and He (Henshaw) antigens to be inherented as Mendelian dominant characters. Hu was found to be associated usually with the *Ns* allele and He associated usually with the *NS* allele of the MNS system. Both antigens were found to be associated less frequently with other MNS alleles. Segregation and recombination have not been observed.

There has been no information to date concerning a relationship, if any, between Hu and He. Recently, however, three generations of a Negro family were found to have members whose erythrocytes reacted with both Hu and He reagents. In addition different Lewis and Js phenotypes were observed.

MATERIALS

The anti-Hunter serum used for this investigation was prepared by immunizing young rabbits (<5 months of age) with red cells of Mr. Hunter, (Landsteiner et al., 1934), whose blood was obtained by Dr. Louis Loeffler of Harlem Valley Hospital, Wingdale, N. Y. The animals were immunized by the Antitoxin Laboratory, New York City Department of Health, Otisville, N. Y. Rabbits immunized with other Hunter positive erythrocytes have consistently failed thus far to produce the desired antibody. Each animal received intravenously 1 ml of a 10 per cent saline suspension of washed erythrocytes daily, four days per week, for a total of eight weeks. Suitable reagents were prepared by absorption of the immune sera with Hu negative erythrocytes. For these absorptions, the sera were diluted 1:5 to 1:30, depending upon the strength of anti-Hu.

The Js serum used for these tests was the second example (Giblett, 1958) and was the sole isoantibody in the serum of Mr. Ro., a hemophiliac (A.H.G. deficiency) immunized by multiple blood transfusions.

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TABLE 1. BLOOD GROUPS OF THE OLYN BY FAMILY

Olyn By Family	Age	Group	M	N	S	s	Hu	He	Js	Le^a	Le^b	rh' C	Rh_0 D	rh'' E	hr' c	hr'' e	hr f
I-1 father	40	0	+	+	+	+	+	+	−	−	−	−	+	+	+	+	+
I-2 mother	37	0	−	+	−	+	+	−	+	−	+	−	+	+	+	+	+
II-1 daughter	6	0	+	+	−	+	−	−	+	−	−	−	+	+	+	−	−
II-2 son	8	0	−	+	+	+	+	+	+	+	−	−	+	−	+	+	+
II-3 daughter	13	0	+	+	−	+	+	−	−	+	−	−	+	+	+	+	+
II-4 daughter	17	0	−	+	+	+	+	+	−	−	+	−	+	+	+	+	+
III-1 son of II-4	>1	0	+	+	+	+	+	+	−	−	−	−	+	−	+	+	+

All positive for Jk^a; all negative for rh_i (Ce), hr^v (V), Lu^a, rh^{w1} (C^w), rh^x (C^x), K, Mi^a, Vw, Wr^a, M^g.

RESULTS

One chromosome of this family appears to bear N, s, and Hu antigens, and another chromosome appears to bear N, S, He and Hu antigens. The blood types of the significant members of this family are shown in Table 1.

The father (I-1) possesses M, N, S and s antigens, and, as a double heterozygote, may have either of two MNS genotypes, *Ms/NS* or *MS/Ns*. The mother (I-2) possesses only the N and s antigens, and also may have either of two MNS genotypes, *Ns/Ns* or Ns/NS^u. The S^u recessive character can be determined only by failure to produce either S or s antigen. S^u cannot be ignored in members of the Negro race because it may have a gene frequency exceeding 4 per cent (Race and Sanger, 1958).

Children II-2 and II-4 inherit S from the father (I-1) along with N, whereas children II-1 and II-3 inherit M from the father but not S. This now establishes the father's MNS genotype as *Ms/NS*. Further information on the mother's MNS genotype is not available.

Children II-2 and II-4, in addition to N and S, also inherit the He antigen from the father. Thus the paternal *NS* allele also produces the He antigen.

Whereas children II-1 and II-3 inherit the father's *Ms* allele, child II-1 lacks the Hu antigen. This shows that the paternal *Ms* allele does not produce Hu. Therefore, the father's *NS* allele must produce the Hu antigen as well as He, unless crossing over has occurred, and there has been no evidence for such an assumption.

A third generation child (III-1) of II-4 also has N, S, Hu and He antigens, and it is entirely likely that this child inherited the maternal *NS* allele transmitted from I-1. Unfortunately the father of this child has refused to have his blood tested.

Two additional children of I-1 and I-2 are known. A daughter, age 22, has not been tested. A son, age 11, was found to be type N but S-negative, and I-1 is thus excluded as his father. Non-paternity could account for the Hu+ He+ persons in this family. However, He+ negroes occur with a frequency of less than 3 per cent (Chalmers et al., 1953), and Hu+ negroes occur with a frequency only 7 per cent (Landsteiner et al., 1934).

Table 1 also demonstrates the independent segregation of genes determining the antigens Js and Lewis. The mother (I-2) is Le(a − b +) and Js +, but the single Le(a − b +) child (II-4) is Js −. Of the two Le(a + b −) children (II-2 and II-3), only II-2 is Js +. One child (II-1) is Le(a − b −) and is Js +, despite the fact that I-2 must have transmitted the *l* allele. Thus Js occurs independently of either the Lewis phenotype or of the expression of *L-l* genes.

DISCUSSION

From data summarized by Race and Sanger (1958), the MN blood group system consists of eleven antigens inherited as dominant characters, some of which contrast with one another. These antigens appear to be determined by a large series of alleles at a single locus similar to the genetic pattern of Rh. However, the opportunity to study the MN system is more restricted because of marked racial differences and an exceedingly low frequency of some antigens.

Although not recorded in the present family, the Mi^a and Vw antigens are known to be closely related. Thus far Vw has not been found to occur in the absence of Mi^a, but Mi^a has been found without Vw (Wallace et al., 1957). Mohn et al. (1958), reported Mi^aVw to be transmitted with the *Ns* allele. They also reported Mi^a (without Vw) to be transmitted with *Ns*, but more frequently with *MS*. Mi^a and Vw have been observed only in Caucasians. Mohn (personal communication) now states that the anti-Mi^a used in his report, contained separable anti-Vw. Therefore, *Ns Vw* was observed rather than *Ns* Mi^a *Vw*.

The Vr antigen (van der Hart et al., 1958) has also been observed only in Caucasians, and found to be transmitted with *Ms*, but not thus far in association with Mi^a or Mi^aVw.

The M^g antigen (Allen et al., 1958) is somewhat different. Although observed in only a single Caucasian family, it was transmitted by an allele which did not bear M, N or S antigens. There is no information as yet concerning s and the M^g allele.

Antisera for S, s, S^U, Mi^a, Vw, Vr and M^g are all of human origin, and attempts to immunize animals have failed. M and N reagents may be of human origin but may also be obtained from lectins (Ottensooser and Silberschmidt, 1953) or from specifically immunized rabbits. Hu and He reagents can be obtained only from rabbits, and Hu has thus far been prepared only from rabbits immunized with the erythrocytes of Mr. Hunter. Rabbit anti-He occasionally results from immunization with He negative erythrocytes (Ikin and Mourant, 1951).

Mourant (1959) is of the opinion that the proportion of Hu positive persons with S^uS^u phenotype might be higher than could be expected by chance. Hu has also been observed frequently with *Ns* and occasionally with *NS* and *Ms*. He is usually carried by *NS*, but less frequently by *Ns*.

The probable existence of an MNS allele which bears both Hu and He antigens indicates the considerable complexity of this blood group system.

Observation of the independent segregation of genes determining the Js and Lewis phenotypes adds to the extensive studies of Giblett and Chase (1959), who showed the independence of *Js* from the ABO, Rh-Hr, MNS, Kell, Duffy, Kidd, Diego and P blood group systems. These investigators failed, however, to

encounter families which would permit linkage counts between *Js* and either *L-l* or Lu^a-Lu^b genes. *Lu* (Lutheran) alone now remains to be studied.

SUMMARY

An allele bearing Hu and He as well as N and S antigens (*NSHuHe*), appears to have been encountered.

In the same family, the gene determining the Js antigen has been observed to have segregated independently of genes determining the Lewis phenotype.

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Problems in Rh Typing as Revealed by a Single Negro Family*

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THE TWO CURRENT THEORIES and notations for the Rh blood group system (Wiener and Wexler, 1959; Race and Sanger, 1958) differ, and neither need be correct when sufficient data become available. The blood typing results of members of a single Negro family reveal Rh alleles which bear patterns of erythrocytic antigens different from those usual in Caucasians. These alleles introduce further difficulties in Rh notation as well as the possibility of serious misinterpretation of serologic tests for disputed paternity.

Attention was first attracted to the By family through a routine genetic investigation which included blood typing. The results of initial Rh tests by the Genetics Laboratory, Department of Biology, Western Reserve University, Cleveland, revealed a mother of type Rh_2Rh_2 (ccDEE) having three type Rh_0 (ccDee) children. Except for one report (Wiener et al., 1952), this result should not be considered possible. The blood types initially encountered in the Charlie By family are shown in Table 1.

In New York, re-examination of the mother revealed that her red cells were very weakly agglutinated by some anti-**rh′** (C) sera and by anti-**hr″** (e) sera when the latter were used in excess and by ficin technic. This permitted phenotypic classification of the mother as Rh_1Rh_2 (CcDEe). The three "type Rh_0" children also proved to be weakly positive for **rh′** (C) with the same antisera, thus becoming Rh_1rh (CcDee) and apparently solving the dilemma.

However, the Rh type of the father attracted interest because of serologic peculiarities of the Negro phenotype, rh′rh (Ccdee), noted by Unger and Wiener (1949), Sturgeon et al. (1958), and Rosenfield (1958). The Charlie By family had one child (II-2) with apparent genotype R^2r', and studies with anti-**hr** (f) and anti-$\mathbf{rh}_i$ (Ce) reagents (Rosenfield and Haber, 1958) revealed that this child was **hr** (f) positive and $\mathbf{rh}_i$ (Ce) negative (Table 2).

The R^2r' genotypes previously studied by Rosenfield and Haber (1958) were clearly positive with anti-$\mathbf{rh}_i$ but negative with anti-**hr**, consistent with the in-

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TABLE 1. INITIAL BLOOD TYPING RESULTS OF THE CHARLIE BY FAMILY

Charlie By Family	age	ABO	rh′ C	Rho D	rh″ E	hr′ c	hr″ e	hrᵛ V	Js	M	N	S	s	Leᵃ	Leᵇ
I-1 father	37	O	+	−	−	+	+	−	+	+	−	+	+	−	+
I-2 mother	39	B	−	+	+	+	−	−	−	+	+	−	+	−	+
II-1 son	17	B	−	+	+	+	+	−	+	+	+	+	+	−	+
II-2 daughter	15	B	+	+	+	+	+	−	+	+	+	+	+	−	+
II-3 daughter	12	O	−	+	+	+	+	−	−	+	+	−	+	−	+
II-4 daughter	10	O	−	+	−	+	+	−	+	+	−	+	+	+	−
II-5 daughter	8	O	−	+	−	+	+	−	−	+	+	+	+	−	+
II-6 son	7	O	−	+	−	+	+	−	−	+	+	+	+	+	−

TABLE 2. RH TYPING OF THE CHARLIE BY FAMILY, USING SEVEN REAGENTS

	rh′ C	Rh₀ D	rh″ E	hr′ c	hr″ e	hr f	rhᵢ Ce
I-1	++	−	−	+++	+++	+++	−
I-2	±	+++	+++	+++	±	−	−
II-1	−	+++	+++	+++	+++	+++	−
II-2	++	+++	+++	+++	+++	+++	−
II-3	−	+++	+++	+++	+++	+++	−
II-4	±	+++	−	+++	+++	+++	−
II-5	±	+++	−	+++	+++	+++	−
II-6	±	+++	−	+++	+++	+++	−

+++ = normal positive results
++ = distinct positive but not as strong as normal
± = questionable result

terpretation that the gene product of r' contained **rh**$_i$ but not **hr**. The reverse result with the R^2r' genotype in the By family (II-2) indicated either that this r' allele was different or behaved differently when paired with an R^2 allele.

To clarify the point and to obtain further information on the peculiar R^1 allele in the By family, some members of the family were tested in titer with a series of Rh-Hr antisera to determine the strength of expression of each Rh-Hr antigen. The red cells for this test, along with suitable controls, were added to aliquots of a master titration of each reagent serum. Agglutination patterns were evaluated by a single observer, and the results were numbered as follows: If the strongest observed agglutination was graded as ++++ and the weakest as ±, ++++ received a value of 9, +++± a value of 8, +++ 7, etc., with ± receiving a value of 2 and doubtful (trace) end-points a value of 1. The final "score" was the sum of all the values contributed by each tube of the titration and the results are shown in Table 3.

Scoring is useful with low avidity Rh-Hr antisera that have titration end-points between 1:16 and 1:128. Duplicate tests usually agree within five per cent, but the result is not worthy of such confidence because it is influenced by too many variables. The method, however, offers the best means now readily available to compare the antigenic strength of a series of bloods that are tested at one time.

Conclusions to be drawn from the data of Table 3 are further restricted be-

TABLE 3. TITRATION SCORES* AS A MEASURE OF RH-HR EXPRESSION IN SOME† MEMBERS OF THE BY FAMILY

Serum No. / Specificity	1 rh′ C	2 rh′ C	3 rh′ C blocked Rh_0 (D)	4 Rh_0 D	5 Rh_0 D	6 rh″ E	7 hr′ c	8 hr″ e	9 hr f
Technic	saline	ficin	saline	saline	ficin	saline	alb.	ficin	ficin
I-1	15	31	18	0	0	0	34	39	36
I-2	0	3	20	44	56	36	23	3	0
II-2	6	33	20	25	61	22	32	33	19
II-3	0	0	0	40	55	47	31	27	14
II-4	0	2	18	39	58	0	18	26	15
II-5	0	2	13	34	60	0	15	22	11
II-6	0	3	18	32	55	0	15	17	11
Caucasian Controls									
Heterozygote	25	43	29	32	48	48	24	26	15
Homozygote	34	55	33	43	50	61	34	31	25

* For method see text

† Child II-1 not tested

Description of Antisera.

1) Anti-**rh′** (c) from a type rh (ccdee) donor. Serum also contained separable anti-$\mathbf{Rh_0}$ (D) but little or no anti-$\mathbf{rh^G}$ (G). The $\mathbf{rh^G}$ antigen is usually associated with $\mathbf{Rh_0}$ and **rh′** antigens but the red cells of rare persons (type rh^G) lack $\mathbf{Rh_0}$ and **rh′** while having $\mathbf{rh^G}$, and thus permit direct testing for anti-$\mathbf{rh^G}$ (Allen and Tippett, 1959). This serum was kindly supplied by Dr. Fred H. Allen, Jr., Blood Grouping Laboratory, Boston, Mass.

2) Anti-**rh′** (C) from a type Rh_2rh donor, contained weak saline agglutinins not active against the cells of the By family. By ficin technic the serum was strong and apparently specific for **rh′**. This also was kindly supplied by Dr. Fred H. Allen, Jr.

3) Anti-$\mathbf{Rh_0}$′ (CD) allegedly specific for **rh′** (C) by saline agglutinin technic because of the presence of blocking antibodies for $\mathbf{Rh_0}$ (D). Actually, most of the antibody was anti-$\mathbf{rh^G}$ as determined by intense reactions with rh^G red cells and inseparability of anti-**rh′** and anti-$\mathbf{Rh_0}$ specificities. Such sera need not be anti-**rh′** but could be anti-$\mathbf{rh^G}$ blocked for $\mathbf{Rh_0}$ and $\mathbf{Rh_0}$-like sites in saline tests. It was kindly supplied by Dr. R. A. Zeitlin, Director, South London Transfusion Centre, Surrey, England.

4) Anti-$\mathbf{Rh_0}$ (D) saline agglutinin from a type rh (ccdee) donor. Serum specific by all technics.

5) Anti-$\mathbf{Rh_0}$ (D) containing albumin and blocking antibodies. Serum specific by all technics.

6) Anti-**rh″** (E) saline agglutinins from a type Rh_1Rh_1 donor. Also contained weak and incomplete **hr′** (c) antibodies, and anti-$\mathbf{hr_1}$ (c-E cross-reaction).

7) Anti-**hr′** (c) albumin active antibodies with weak saline agglutinins, from a type Rh_1Rh_1 donor. Negative with Rh_zRh_1 test cells. Anti-**hr** (f) could not be separated.

8) Anti-**hr″** (e) from a type Rh_2Rh_2 donor. Most active with ficin treated red cells. No evidence of anti-**hr** (f) with type $\bar{R}h_0$ (cD-f) test red cells.

9) Anti-**hr** (f). Same as that of original report (Rosenfield et al, 1953).

cause they apply to only a single family; other pedigrees with similar blood types might reveal differences. Nevertheless, these results warrant critical examination because they are unusual.

In no instance for **rh′** (C) did a member of this family score as a "usual heter-

ozygote", and it is possible that all are **rh′** negative. Except for II-3, they could possess either of two as yet unrecognized antigens (one determined by the father's allele, the other by the mother's) which resemble **rh′**, perhaps analogous to $\mathbf{rh}^{w1}$ (C^w). Because blood typing laboratories will recognize the Negro phenotype, rh′rh, as **rh′** positive, both I-1 and II-2 could be thought of as **rh′** positive, though weakly so. But the **rh′** reactions of the other members of the family, especially with "pure" anti-**rh′** reagents (sera No. 1 and 2), are not consistent with **rh′** positive classification.

The phenotype of II-3 is typical of Rh_2rh and the R^2r genotype. The mother, I-2, however, not only fails to score in "homozygous dosage" with **rh″** (E), but lacks the **rh″** expression of a "heterozygote". This should (as suspected from the earl er blood typings) exclude the possibility of this person being Rh_2Rh_2 (cf Table 2). The allele that is paired with R^2 in the mother may even have a slight suppressive effect on the expression of **rh″** (e.g., gene interaction at the phenotypic level), similar to the effect of R^1 on R^2 (Lawler and Race, 1950). In II-2, where **rh″** scores considerably less than expected of a normal heterozygote, the paired r' allele appears to have a distinctly greater suppressive effect on **rh″** determined by R^2.

If I-1 and II-2 are **rh′** positive, they should not score higher than "heterozygotes" for **hr′** (c). It is clear, however, that they score as "homozygotes", just like the R^2r child (II-3). The other members of the family, all with little or no **rh′** representation on their red cells, score only as "heterozygotes" for **hr′**. Therefore, one allele thought of as r' (*Cde*) appears to bear both **rh′** and **hr′**, whereas another allele which bears essentially no **rh′** is found not to bear **hr′** at all, unless both these antigens are suppressed by either R^2 or r alleles with which the determining allele is paired in this family.

Of further interest is the **hr″** (e) and **hr** (f) status. Although the mother (I-2) does not have a "double dose" of **rh″**, she lacks any significant expression of **hr″**, and has no expression of **hr**. This is consistent with the observation that children II-3, II-4, II-5 and II-6 all score as expected of "heterozygotes" for **hr″** and **hr**. The father (I-1) scores in excess of a "homozygote" for both **hr″** and **hr**, and the daughter (II-2) inheriting his r' allele, scores in excess of a "heterozygote" for both these antigens. In each instance the excess is similar in father and daughter. Therefore, not only does the r' in this family bear **hr**, but it bears what appears to be an excessive amount of this antigen and of the **hr″** antigen as well, as shown in two genotypes, $r'r$ and R^2r' (I-1 and II-2).

Scores with anti-$\mathbf{Rh_0}$ (D) reagents failed to demonstrate any unusual expression of this antigen, such as would be expected of $\bar{\bar{R}}^o$ (*-D-*) alleles. Furthermore,

TABLE 4. ANTIGENS DETERMINED BY NEGRO "r'" COMPARED WITH ANTIGENS DETERMINED BY CAUCASIAN r' (Cde) AND r (cde) ALLELES

	rh′ C	**Rh_0** D	**rh″** E	**hr′** c	**hr″** e	**hr** f	**rh_i** Ce
Negro "r'"	++	−	−	+++	++++	++++	−
Caucasian r'	+++	−	−	−	+++	−	+++
Caucasian or Negro r	−	−	−	+++	+++	+++	−

TABLE 5. COMPARISON OF THE ANTIGENS DETERMINED BY R^1 (*CDe*) AND $\bar{R}^o$ (*-D-*) ALLELES WITH THE ANTIGENS DETERMINED BY THE SO-CALLED "R^1" ALLELE OF THE BY FAMILY

	rh′ C	**Rh₀** D	**rh″** E	**hr′** c	**hr″** e	**hr** f	**rh$_i$** Ce
R^1	+++	+++	−	−	+++	−	+++
$\bar{R}^o$	−	++++	−	−	−	−	−
By "R^1"	±	+++	−	−	±	−	−

II-2 scores low with saline agglutinating anti-**Rh₀**, consistent with the possibility that the r′ allele of this R^2r' genotype might partly suppress the **Rh₀** antigen determined by the paired R^2 allele (Ceppellini, Dunn and Turri, 1955).

In Table 4 the antigens determined by the *r′* allele of this family are compared with the usually accepted antigens determined by Caucasian *r′* and *r* alleles.

The By *r′* allele appears to resemble *r* rather than *r′*. It differs from *r* in bearing a weak **rh′** (C) antigen and an excessive amount of **hr″** (e) and **hr** (f) antigens. It differs even more strikingly from *r′* in bearing **hr′** (c) and **hr** (f) while failing to bear the expected **rh$_i$** (Ce) antigen. Nevertheless because alleles are ordinarily classified on the basis of **rh′**, **Rh₀**, and **rh″** (C, D and E) antigens, the By *r′* allele would be classified as *r′* or variant *r′* by most laboratories, and r'^n (*C^{n}de*) suggested by Sturgeon et al. (1958) remains a suitable term.

In Table 5 the antigens determined by the mother's so-called "R^1" allele are compared to the usual antigens of R^1 (*CDe*) and $\bar{R}^o$ (*-D-*) alleles. The term $\bar{R}^o$ has been used at the suggestion of Wiener (personal communication) to designate an Rh allele which produces **Rh₀** but not the antigens **rh′**, **hr′**, **rh″** or **hr″** (*-D-*). Similarly, the term $\bar{R}^o$ would indicate an allele which would produce **hr′** but not **rh′**, **rh″** or **hr″** (cD-). This is the subject of another communication now in preparation.

The antigens determined by the By "R^1" allele are thus seen to bear close resemblance to the antigens determined by $\bar{R}^o$ alleles. For this reason, the designation $\bar{R}^N$, is suggested. The allele differs from $\bar{R}^o$, in producing small amounts of **rh′** and **hr″** and failing to produce an unusual amount of **Rh₀**.

If the *r′* alleles in Negro population samples bear both **rh′** (C) and **hr′** (c) antigens (i.e., are all r'^n), statistical analysis of available data might indicate this. Mourant (1954) lists 15 Negro population samples obtained from Africa and New York City, each of which contains rh′rh (Ccdee) individuals. In none of these samples is the rh′rh′ phenotype observed. Two isolates of Carib Negroes in British Honduras have been blood typed, and each has a relatively high frequency of rh′rh but no observed rh′rh′ phenotypes. Populations from Charleston (Pollitzer, 1958), Seattle (Giblett et al., 1957), New York (De Natale et al., 1955) and Africa (De Natale et al., 1955) also contain rh′rh but no rh′rh′.

By usual assumption, the rh′rh (Ccdee) phenotype class consists only of *r′r* (*Cde/cde*) heterozygotes, whereas rh′rh′ (CCdee) phenotypes are comprised of only *r′r′* (*Cde/Cde*) homozygotes. Without family data, *r′* cannot be recognized in other phenotypes by ordinary blood typing technic. Therefore the frequency

TABLE 6. ABSENCE OF rh′rh′ (ccdee) IN NEGRO POPULATIONS

Population Sample		Number tested	Observed rh (ccdee)	Observed rh′rh (Ccdee)	r′r′ Calculated from $\bar{r'} = \overline{rh'rh}/2\bar{r}$	r′r′ Genotppes from $\bar{r'} = \sqrt{\overline{rh'} + \overline{rh}} - \bar{r}$
Referene numbers from Mourant (1954)	1230	644	9	5	.697	.553
	646	267	10	2	.102	.091
	646	127	6	1	.043	.038
	658	104	9	1	.029	.026
	251	145	5	1	.051	.046
	251	106	6	1	.041	.038
	251	124	7	1	.035	.033
	251	165	3	1	.083	.073
	62	161	9	6	1.015	.764
	21	124	7	1	.122	.102
	21	172	7	1	.035	.021
	190	133	8	1	.033	.029
	1455	284	17	2	.056	.056
Charleston[1]		515	34	6	.265	.243
Seattle[2]		327	27	3	.098	.079
New York[3]		168	10	1	.040	.024
Africa[3]		150	8	1	.032	.029
Honduras[4]		740	57	22	2.126	1.791
Honduras[4]		150	9	1	.028	.031
		4919	261	60	5.073	4.190

From 4,919 individuals tested, if 5.073 $r'r'$ genotypes are expected and no rh′rh′ phenotypes are encountered, $\chi_1^2 = 5.69$ and $p < 0.02$. If 4.190 $r'r'$ genotypes are expected, $\chi_1^2 = 4.82$ and $p < 0.03$.

[1] Pollitzer, 1958.
[2] Giblett et al., 1957.
[3] De Natale et al., 1955.
[4] Rosenfield and Firschein, unpublished observations.

of r' must be calculated from the frequency of rh′ phenotypes. Two formulae are commonly used (Mourant, 1954):

(1) *frequency rh′rh*/2 × $\sqrt{\textit{frequency rh}}$

if rh′rh is $r'r$ then $\overline{rh'rh} = 2(\bar{r'} \times \bar{r})$ and $\bar{r'} = \overline{rh'rh}/2\sqrt{\overline{rh}}$

(2) $\sqrt{\textit{frequency rh′rh} + \textit{frequency rh′rh′} + \textit{frequency rh}}$ *minus* $\sqrt{\textit{frequency rh}}$

if $$\sqrt{\overline{r'r'} + 2\overline{r'r} + \overline{rr}} = (\bar{r'} + \bar{r})$$

and if $$\overline{rh'rh'} = \overline{r'r'}, \quad \text{and } \overline{rh'rh} = 2\overline{r'r}$$

then $$\sqrt{\overline{rh'rh'} + \overline{rh'rh} + \overline{rh}} = \bar{r'} + \bar{r}$$

and $$\bar{r'} = \sqrt{\overline{rh'rh'} + \overline{rh'rh} + \overline{rh}} - \sqrt{\overline{rh}}$$

If r' (*Cde*) bears **rh′** (C) but not **hr′** (c), r' should be calculated by formula No.

1. It may, however, be calculated by formula No. 2 but this yields values independent of **hr′** (c) results. Table 6 lists these populations and both values, and compares the absence of rh′rh′ phenotypes with the total $r'r'$ genotypes expected by each formula. This difference is seen to be significant at the 2% level. In Caucasian populations, rh′rh′ phenotypes, although rare, are encountered as expected (Mourant, 1954).

The By $\bar{\bar{R}}^N$ allele cannot be estimated as readily in population blood typing results, because anti-**rh′** (C) reagents vary in their reactions with these gene products. The samples in two published populations, Wiener et al. (1952) and Pollitzer (1958), may contain these alleles. Wiener et al. noted (1) an individual of phenotype Rh_1rh whose red cells reacted doubtfully with anti-**hr″**, and (2) a mother of type Rh_0 who had a child of type Rh_2Rh_2. Pollitzer's data differ from Hardy-Weinberg equilibrium unless alleles of two varieties, $\bar{\bar{R}}^o$ (*-D-*) and $\bar{R}^o$ (*cD-*), are postulated.

Relatives of the Charlie By family were studied to obtain further information about the r'^n and $\bar{\bar{R}}^N$ alleles. Forty individuals in this pedigree were examined and the blood typing results are summarized in Table 7. The Charlie By family of Tables 1, 2, and 3 is Family I-3 in Table 7. Family I-2 has been reported separately as the Olyn By family, in which a chromosome was observed which appeared to bear N, S, Hu and He antigens through three generations; Family I-2 also showed independent segregation of genes determining Js and Lewis (Rosenfield et al., 1960). The r'^n and $\bar{\bar{R}}^N$ alleles could be recognized in relatives of Family I-3 by unusual results with selected Rh-Hr reagents as described. Family III, like Family I-3, disclosed $\bar{\bar{R}}^N r$ or $\bar{\bar{R}}^N R^o$ children of a parent with $\bar{\bar{R}}^N R^2$ genotype.

DISCUSSION

A Negro family has revealed two unusual Rh alleles. One allele that would, ordinarily, be classified as "r'", appeared to bear **hr′** (c) as well as **rh′** (C), and to bear **hr** (f) rather than **rh**$_i$ (Ce). Although its gene products are similar to those of usual r alleles, the term "r'^n" (Sturgeon et al., 1958) is preferred. The second unusual allele, that might sometimes be over-classified as "R^1", has been found to bear only a questionable amount of **rh′** (C) and **hr″** (e). Because of the similarity of its antigens to those of $\bar{\bar{R}}^o$ alleles, the term $\bar{\bar{R}}^N$ has been suggested. $\bar{\bar{R}}^N$ was not associated with an excessive expression of **Rh$_0$** shown by $\bar{\bar{R}}^o$ alleles.

Both of these alleles raise questions about the validity of some serologic tests for disputed paternity in Negroes. A mating of the phenotypes Rh_1rh (CcDee) and rh (ccdee) might have children of phenotype Rh_1rh and rh′rh if the Rh positive parent happened to be of $R^1r'^n$ genotype. Although this genotype has not yet been identified, its serologic properties might not differ appreciably from other genotypes comprising the Rh_1rh phenotype. The absence of rh′rh′ phenotypes in available population studies of Negro Rh-Hr phenotypes, supports this view.

On the other hand, the $\bar{\bar{R}}^N$ allele is an obvious source of difficulty, typical examples of which are illustrated in the By family (cf Families I-3 and III).

TABLE 7.

	Age	ABO	rh′ C	Rh$_o$ D	rh″ E	hr′ c	hr″ e	hr f	rh$_i$ Ce	hr^V V	M	N	S	s	Hu	He	Js	Le^a	Le^b	Fy^a
FAMILY I																				
father[1]	66		not tested																	
mother	dead																			
1. son	42		see father Family I-1																	
2. daughter	41		not tested																	
3. son	40		see father Family I-2																	
4. daughter	39		not tested																	
5. daughter	38		not tested																	
6. son	37		see father Family I-3																	
7. daughter	31	A$_1$	+	−	−	+	+	+	−	−	+	−	−	+	−	−	+	not done		−
8. daughter	?		not tested																	
9. daughter	26		see mother Family I-4																	
10. son	23		not tested																	
FAMILY I-1																				
father[2]	42	A$_1$	+	−	−	+	+	+	−	−	+	−	+	+	−	−	−	not done		−
mother[3]	42	O	+	+	−	+	+	+	+	−	−	+	−	+	+	−	−	−	+	−
1. daughter	25		not tested																	
2. son	6	O	+	+	−	+	+	+	+	−	+	+	−	+	−	−	−	−	+	−
3. son[4]	5	O	+	+	−	+	+	+	+	−	−	+	−	+	−	−	−	−	+	−
4. son	4	O	−	+	−	+	+	+	−	−	+	+	−	+	−	−	−	−	−	−
5. daughter	2		not tested																	
6. daughter	<1		not tested																	
FAMILY I-2[5]																				
father[6]	40	O	−	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	−	−
mother	37	O	−	+	+	+	+	+	−	−	−	+	−	+	+	−	+	−	+	−
1. daughter	22		not tested																	
2. daughter	17	O	−	+	+	+	+	+	−	−	−	+	+	+	+	+	−	−	+	−
3. daughter	13	O	−	+	+	+	+	+	−	−	+	+	+	+	+	−	−	+	−	−
4. son[7]	11	A$_1$	−	+	+	+	+	+	−	−	−	+	−	+	−	−	−	+	−	+

5. son	8	O	−	+	−	+	+	+	−	−	−	+	+	+	+	+	+	+	−	−
6. daughter	6	O	−	+	+	+	−	−	−	−	+	+	−	+	−	−	+	−	−	−
FAMILY I-2A																				
father			not tested																	
mother	see daughter No. 2, Family I-2																			
1. son[8]	<1	O	−	+	−	+	+	+	−	−	−	+	+	+	+	+	−	−	−	−
FAMILY I-3 (Family shown in tables 1, 2, and 3.																				
father[9]	37	O	+	−	−	+	+	+	−	−	+	−	+	+	−	−	+	−	+	−
mother[10]	39	B	±	+	+	+	±	−	−	−	+	+	−	+	−	−	−	−	+	−
1. son	17	B	−	+	+	+	+	+	−	−	+	−	+	+	−	−	+	−	+	−
2. daughter	15	B	+	+	+	+	+	+	−	−	+	+	+	+	−	−	+	−	+	−
3. daughter	12	O	−	+	+	+	+	+	−	−	+	+	−	+	−	−	−	−	+	−
4. daughter	10	O	±	+	−	+	+	+	−	−	+	−	+	+	−	−	+	+	−	−
5. daughter	8	B	±	+	−	+	+	+	−	−	+	+	+	+	−	−	−	−	+	−
6. son	7	B	±	+	−	+	+	+	−	−	+	+	+	+	−	−	−	+	−	−
7. son[11]	4	O	±	+	−	+	+	+	−	−	−	+	−	+	−	−	−	+	−	−
8. son[11]	2	B	±	+	−	+	+	+	−	−	+	+	−	+	−	−	−	−	+	−
FAMILY I-4																				
father			not tested																	
mother[12]	26	O	−	+	−	+	+	+	−	−	+	−	+	+	−	−	+	+	−	−
1. daughter	7	O	−	+	−	+	+	+	−	−	+	−	−	+	−	−	−	+	−	−
2. daughter	6	O	−	+	−	+	+	+	−	−	+	−	−	+	−	−	+	+	−	−
3. son	4	O	−	+	−	+	+	+	−	−	+	−	−	+	−	−	−	+	−	−
FAMILY II																				
father[13]	45	O	−	+	−	+	+	+	−	+	−	+	−	+	−	−	−	−	+	−
mother	42	O	−	+	+	+	+	+	−	−	+	+	+	+	+	+	−	−	+	−
1. daughter	23		see mother, Family II-1																	
2. son	21	O	−	+	−	+	+	+	−	+	−	+	−	+	+	−	−	−	+	−
3. daughter	19	O	−	+	+	+	+	+	−	+	−	+	−	+	+	−	−	−	−	−
FAMILY III-1																				
father			not tested																	

TABLE 7—*Continued*

	Age	ABO	rh′ C	Rh$_0$ D	rh″ E	hr′ c	hr″ e	hr f	rh$_i$ Ce	hr^v V	M	N	S	s	Hu	He	Js	Le^a	Le^b	Fy^a
mother[14]	23	O	−	+	+	+	+	+	−	+	−	+	−	+	+	−	−	−	+	−
1. daughter	4	B	−	+	+	+	+	+	−	+	−	+	−	+	−	−	−	−	+	−
2. daughter	2	O	−	+	−	+	+	+	−	+	−	+	−	+	−	−	−	−	+	−
FAMILY III																				
father[15]	35	O	±	+	+	+	±	−	−	−	+	+	−	+	−	−	−	not done		not d.
mother	36	O	−	+	+	+	+	+	−	+	−	+	−	+	−	−	−	not done		not d.
1. son	15	O	±	+	+	+	±	−	−	−	+	+	−	+	−	−	−	+	−	+
2. son	12	O	±	+	−	+	+	+	−	+	−	+	−	+	−	−	−	+	−	−
3. daughter	11	O	−	+	+	+	−	−	−	−	−	+	−	+	−	−	−	not done		not d.
4. daughter	7	O	±	+	−	+	+	+	−	+	+	+	−	+	−	−	−	not done		not d.
5. daughter	3		not tested																	
6. daughter	<1		not tested																	

All positive for Jk^a.
All negative for K, Lu^a, Mi^a, and M^g.
[1] Brother of father, Family II.
[2] Child No. 1, Family I.
[3] Sister of mother, Family I-2.
[4] Excluded paternity on basis of MNS.
[5] Reported separately for Hu, He, Js and Le information.
[6] Child No. 3, Family I.
[7] Excluded paternity on basis of ABO, Fy^a, and MNS.
[8] Newborn, Lewis status in doubt.
[9] Child No. 6, Family I.
[10] Sister of father, Family III.
[11] Admittedly extramarital.
[12] Child No. 9, Family I.
[13] Brother of father, Family I.
[14] Child No. 1, Family II.
[15] Brother of mother, Family I-3.

Only by careful serologic tests can this allele be detected, and it should be suspected whenever presumptively type Rh_0 children are encountered from Rh_1Rh_1 or Rh_2Rh_2 parents, or when Rh_0 parents appear to have Rh_1Rh_1 or Rh_2Rh_2 children.

A possible example of the latter possibility was reported by Wiener et al. (1952). During the course of serologic tests for disputed paternity, a mother of Rh_0 phenotype was observed to have a child of Rh_2Rh_2 phenotype. To explain this family, Wiener invoked a hypothetical allele, R^{o_u}, bearing **Rh$_0$** (D) and **hr′** (c), but neither **rh″** (E) nor **hr″** (e). Wiener did not attempt to quantitate the strength of **hr′** (c) expression on the red cells of either the mother or the child in this family, and there is no way of knowing if the serologic problem he reported is identical with that now described.

Very recently, however, Witebsky and Wiener (personal communication) encountered a similar Negro family, and titration with **hr′** and **hr″** reagents did yield the results expected either of the allele $\bar{\bar{R}}^o$ (*-D-*) or of the allele $\bar{\bar{R}}^N$.

	Phenotype	Gene Dose hr′	Gene Dose hr″
Father	$Rh_1{}^w Rh_1$	0	double
Mother	$\bar{\bar{R}}h_0rh$	single	single
Child	$Rh_1{}^w\bar{\bar{R}}h_0$	0	single

The frequency of the r'^n allele may be as high as 6% of the Negro gene pool (cf Table 6). The frequency of $\bar{\bar{R}}^N$ cannot be calculated but it might be as high as 2% and not have attracted attention through disturbance of the usual tests for Hardy-Weinberg equilibrium.

In a previous report (Rosenfield and Haber, 1958) the term **rh$_i$** (Ce) was introduced to describe an antigen carried by R^1 and r' alleles. The same report considered the antigen f (Rosenfield et al., 1953) as **hr** (ce) and carried by R^o and r alleles. The r'^n allele appears to bear **rh′** (C) and **hr′** (c), but not **rh$_i$** (Ce). On the other hand, the same allele appears to bear both **hr′** (c) and **hr″** (e) and, as expected, bears **hr** (ce). The **rh′** (C) factor arising from the r'^n allele is feeble in comparison with that arising from usual R^1 and r' alleles (cf Table 3). This **rh′** (C) antigen actually may be considerably different to account for the failure of appearance of an expected **rh$_i$** (Ce) antigen. Some other explanations were considered in an earlier communication (Sanger et al., 1953), but further data will be required before the relationship of Rh-Hr antigens are better understood.

Future studies of Negro families may yield information slightly different from that now reported. Perhaps all r'^n alleles in Negroes are not identical, or further variations of $\bar{\bar{R}}^N$ alleles may be encountered. Evidence for $\bar{R}^o$ (*cD-f*) and $\bar{\bar{\mathfrak{R}}}^o$ ($cD^u(e)\ f$) (to be reported) has been found already.

An assumption that Rh-Hr antigens are equally expressed by different alleles, or that all of the gene products of the same allele are equally expressed in different genotypes, is not supported by available data. The results of Lawler and Race (1950) as well as those of Race, Sanger, Levine, van Loghem, van der Hart and Cameron (1954) are consistent with an interpretation that Rh-Hr antigens vary in different gene products. Ceppellini, Dunn and Turri (1955),

Chown and Lewis (1957), and Levine, Celano, McGee, Muschel, and Griset (1957) demonstrated the suppression of **Rh_0** (D) by an allele paired with either r' or r^y. Such gene interaction is probably not restricted to **Rh_0** expression. Data consistent with an effect of gene interaction on the expression of **hr′** (c) may be found in the report of McGee, Levine and Celano (1957). In the present report, possible suppressive effect of r'^n and $\bar{\bar{R}}^N$ on the **rh″** determined by paired R^2 has been mentioned (cf Table 3). It is to be hoped that semi-quantitation of Rh-Hr antigens in a large series of genotypes may permit recognition of new differences in these antigens and their determining alleles, and a broader concept of the role of gene interaction in this system.

SUMMARY

Routine Rh-Hr typing of a Negro family revealed a mother of phenotype Rh_2Rh_2 (ccDee) with three children of phenotype Rh_0 (ccDee). Tests with additional reagents and semi-quantitation of Rh-Hr antigen expression by means of titration scores, revealed an Rh allele, $\bar{\bar{R}}^N$, bearing only **Rh_0** (D) in normal expression. This allele was found to differ from $\bar{\bar{R}}^o$ (*-D-*), by producing traces of **rh′** (C) and **hr″** (e) as defined by some reagents, and failing to produce excessive **Rh_0** (D). Such an allele introduces a significant source of error into serologic tests for disputed paternity involving Negroes.

This family also had the r'^n allele ("Negro" r'). The same serologic analysis showed that this allele produces **hr** (f) antigen rather than **rh_i** (Ce), and seemed to produce both **rh′** (C) and **hr′** (c) antigens. This pattern of antigens, except for **rh′** (C), is similar to that produced by r rather than r' alleles. Analysis of available Negro population samples, revealed a significant ($p < .02$) absence of rh′rh′ phenotypes, consistent with the hypothesis that all, or almost all, r' alleles in Negroes are r'^n, bearing both **rh′** (C) and **hr′** (c) antigens. This finding introduces a further source of error into Rh-Hr serologic tests for disputed paternity in Negroes.

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The Inheritance of Serum Haptoglobin Types in American Negroes: Evidence for a Third Allele Hp^{2m}

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SMITHIES (1955) REPORTED that when human serum is subjected to electrophoresis in starch gel, haptoglobin (that portion of the alpha-2 globulin which binds hemoglobin) differentiates into one of three distinct patterns. Smithies and Walker (1956) showed that these patterns are under the control of two allelic autosomal genes called Hp^1 and Hp^2, the resultant phenotypes being Hp 1-1, Hp 2-2 and Hp 2-1. Further support for this mechanism of inheritance was provided by Galatius-Jensen (1958a) in a study of 205 families.

Subsequently, Connell and Smithies (1959) and Giblett (1959) described a variant of the Hp 2-1 pattern called Hp 2-1 (mod). As shown in Figure 1, this pattern is characterized by a heavy concentration of the two fastest-moving components of type 2-1, the remaining bands being absent or scarcely discernible. Although it is present in the serum of about ten per cent of American negroes, it occurs rarely in the other ethnic groups so far tested. Harris, Lawler, Robson and Smithies (1959) reported a Scotch-Canadian kinship containing this modified type, and postulated that it might be due either to a third allele, Hp^{2m}, in combination with Hp^1, or to a dominant modifying gene at another locus.

The Hp 2-1 (mod) pattern is distinctly different from that of the rare aberrant type described by Galatius-Jensen (1958b) or the so-called "Johnson type" found, thus far, in only two individuals within a single family (Giblett, in preparation).

The phenomenon of "ahaptoglobinemia", or the apparent absence of haptoglobin, was shown by Nosslin and Nyman (1958) to be a common finding in patients with various kinds of hemolytic disease (i.e. with increased red cell destruction). Galatius-Jensen (1958b) reported that ahaptoglobinemia occurs in considerably less than one percent of apparently healthy Danish adults. However, in children between 4 months and 15 years, the incidence is 1.6 per cent;

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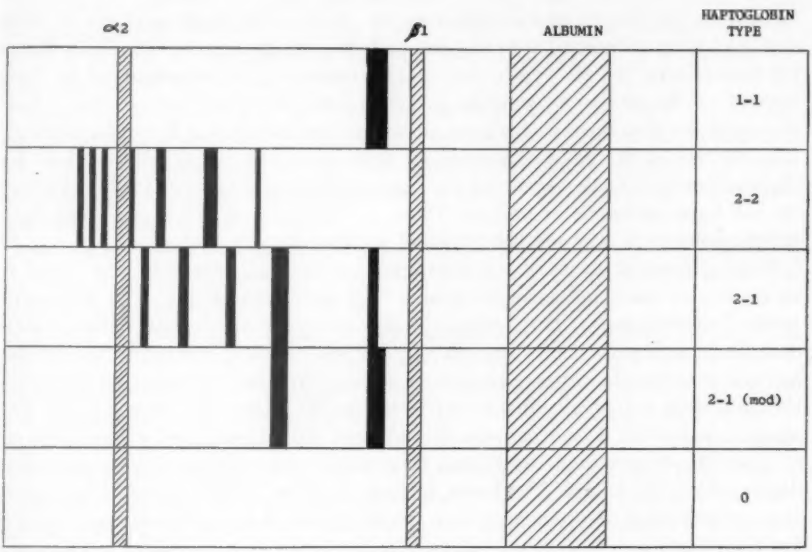

FIG. 1. Diagrammatic representation of the various haptoglobin patterns discussed in this paper, as shown by starch gel electrophoresis.

Haptoglobin ■

Non-haptoglobin protein ▨

whereas it is over 90 per cent in newborn infants. Allison, Blumberg and ap Rees (1958) found no demonstrable haptoglobin in the serum of 32 out of 99 Nigerians. They postulated a genetic basis for this phenomenon, designating it as a new type, Hp 0-0. Giblett (1959) reported apparent ahaptoglobinemia in slightly less than 5 per cent of 406 American Negro blood donors. Galatius-Jensen (1958b) and Harris, Robson, and Sinescalco (1958) each reported a white family in which some members had no visible serum haptoglobin. It is suggested in both reports that ahaptoglobinemia might be explained by the existence of a "silent" allele at the Hp locus, or by the activity of a modifying or suppressor gene.

The present paper reports an investigation of the haptoglobin types in 92 American Negro families. These data are then utilized to delineate the mechanism of inheritance of the phenotypes Hp 2-1 (mod) as well as Hp 0 (ahaptoglobinemia).

MATERIALS AND METHODS

Specimens of blood drawn into tubes containing crystalline EDTA were obtained from over 500 members of 92 Negro families living in Cleveland and Seattle. In most instances, these families were selected on the basis of the presence of the sickle hemoglobin gene in at least one parent, as a part of a separate study on gene linkage being conducted by one of us (A.G.S.). The Hb type, ABO,

MNS, Rh, K, Fy, P, and Js blood types, the Gm-like type, and the secretor status of each individual were determined. We are grateful to Dr. John Harris for determining the Hb types. Any children shown to be extramarital by these tests or by the mother's admission (a not uncommon event) were excluded from the study, as were those with ahaptoglobinemia secondary to a hemoglobinopathy (i.e., Hb SS or SC disease) associated with increased red cell destruction. Individuals with a single abnormal hemoglobin gene were not excluded, since they do not have increased hemolysis. Thus, a correlation of Hb S or C trait with ahaptoglobinemia was neither expected nor found.

Hemoglobin, in excess of the haptoglobin-combining capacity, was added to each plasma specimen prior to electrophoresis in starch gel. The horizontal method of Smithies (1955), using the discontinuous tris-borate buffer system described by Poulik (1957) proved very satisfactory for separating the plasma protein components. After eight hours of electrophoresis at room temperature, the gels were cut longitudinally into two similar slices. One was stained with amido black B10. The other was first stained with benzidine for identification of the hapto-hemoglobin bands, and then (after thorough washing) immersed in dilute amido black B10. The hemoglobin-containing bands, colored black, were thus permanently distinguished from the blue colored protein bands not containing hemoglobin.

RESULTS AND ANALYSIS

The haptoglobin types of the Negro family members are summarized in Table 1.

TABLE 1. HAPTOGLOBIN TYPES OF PARENTS AND CHILDREN IN 92 NEGRO FAMILIES

Parents	Children						
	No.	1-1	2-2	2-1	2-1M*	0**	Totals
1-1 × 1-1	8	37	0	0	0	1	38
1-1 × 2-2	7	0	0	27	2	1	30
1-1 × 2-1	15	30	0	16	0	2	48
1-1 × 2-1M	6	7	0	0	4	4	15
1-1 × 0	2	1	0	0	1	4	6
2-2 × 2-2	1	0	1	0	0	0	1
2-2 × 2-1	19	0	34	18	1	4	57
2-2 × 2-1M	2	0	4	5	0	3	12
2-2 × 0	1	0	0	0	0	2	2
2-1 × 2-1	13	8	11	12	0	4	35
2-1 × 2-1M	10	10	4	9	7	6	36
2-1 × 0	3	1	0	3	0	2	6
2-1M × 2-1M	4	3	1	0	8	5	17
2-1M × 0†	1	1	1	2	0	0	4
0 × 0	0	0	0	0	0	0	0
Totals	92	98	56	92	23	38	307

* 2-1M refers to haptoglobin type 2-1 (mod)
** 0 refers to apparent ahaptoglobinemia
† This parent has Hb SC

Among the parents, at least one example of all possible haptoglobin type combinations was obtained except two ahaptoglobinemics (i.e. mating Hp 0 × Hp 0). However, in the single instance of a Hp 2-1 (mod) × Hp 0 mating, the ahaptoglobinemic father had Hb SC disease.

In general, the data in Table 1 confirm the hypothesis that two alleles, Hp^1 and Hp^2 are responsible for determining the haptoglobin patterns Hp 1-1, Hp 2-2 and Hp 2-1, representing the two homozygotes and one heterozygote, respectively. However, an additional explanation is needed to account for the phenotypes Hp 2-1 (mod) and Hp 0.

Analysis of the data suggests that Hp 2-1 (mod) is almost certainly, and Hp 0 is usually, a variant expression of Hp 2-1. The following is a presentation of this analysis and an explanation of the source of the variations.

Figure 2 is a pedigree of a family representing three generations. The mating of II-1, who is Hp 1-1, and II-2 ,who is Hp 0, yielded an Hp 1-1 and an Hp 2-1 (mod) child as well as an Hp 0 child. Clearly, the ahaptoglobinemia of II-2 must be a phenotypic variant of Hp 2-1 (mod). This allows a tentative consideration of the phenotype Hp 0 as a variant of Hp 2-1 (mod) and excludes the hypothesis that Hp 0 is due to homozygosis for a recessive allele (Hp^0). The eight children in generation II who represent all of the Hp phenotypes except Hp 2-1 lend further support to this view. The fact that three of them have ahaptoglobinemia demonstrates that ahaptoglobinemia is not due to a recessive allele (Hp^0) at the Hp locus, nor to a dominant suppressor with complete penetrance.

In matings which are expected to segregate for at least two of the three phenotypes (Hp 1-1, Hp 2-2 and Hp 2-1), the ratios among the offspring approach the Mendelian expectancies only if the phenotypes Hp 2-1, Hp 2-1 (mod) and Hp 0 are combined. This is demonstrated in Table 2, where the data are grouped to test the segregation of homozygotes (Hp 1-1 and Hp 2-2) vs. presumed heterozygotes (Hp 2-1, Hp 2-1 (mod) and Hp 0). The grand totals are close to the expected 1:1 ratio. However, for reasons which are not clear, the distributions in

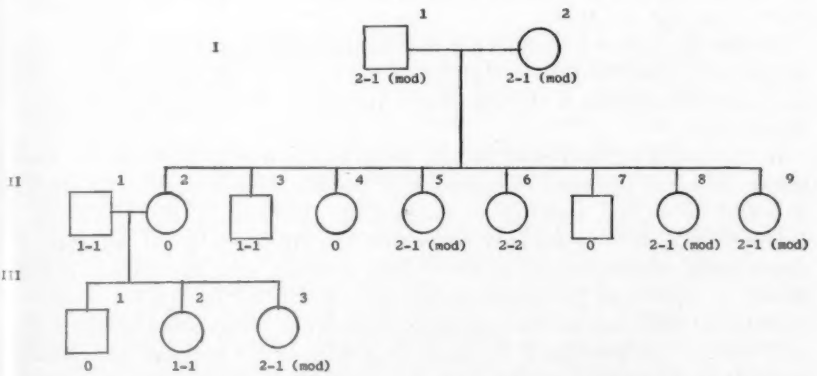

FIG. 2. Pedigree of family showing inheritance of the phenotypes Hp 2-1 (mod) and Hp 0.

TABLE 2. HAPTOGLOBIN PHENOTYPE DISTRIBUTION OF HOMOZYGOUS AND PRESUMED HETEROZYGOUS OFFSPRING FROM VARIOUS MATINGS

	Mating	Offspring		Totals
		1-1 + 2-2	2-1 + 2-1M + 0	
(A)	1-1 × 2-1	30	18	48
	2-1 × 2-1	19	16	35
	2-2 × 2-1	34	23	57
	Subtotals	83	57	140
(B)	1-1 × 2-1M	7	8	15
	1-1 × 0	1	5	6
	2-2 × 2-1M	4	8	12
	2-2 × 0	0	2	2
	2-1 × 2-1M	14	22	36
	2-1 × 0	1	5	6
	2-1M × 2-1M	4	13	17
	Subtotals	31	63	94
	Grand totals	114	120	234

the subtotals, based (A) on those matings in which neither parent was Hp 2-1 (mod) or Hp 0 and (B) on those matings in which at least one parent was of these phenotypes, differed significantly from 1:1 ratios, and in opposite directions [$\chi^2_{(1)} = 4.83$: $P < .05$ for group (A) and $\chi^2_{(1)} = 10.894$; $P < .001$ for group (B)]. There is an excess of the phenotypes corresponding to the homozygous genotypes in group (A) and a deficit of these phenotypes in group (B).

Insofar as the total sample in this study is concerned, the data are consistent with the assumption that Hp 2-1 (mod) always, and Hp 0 usually, represent variant expressions of Hp 2-1. The change in the phenotype may be due to (1) a modifier on another chromosome, (2) a modifier on the same chromosome, linked to the Hp locus but not absolutely (since an absolutely-linked modifier is simply another allele) or (3), an allele at the Hp locus which, in the heterozygous state, has variable expressivity yielding the phenotypes Hp 2-1, Hp 2-1 (mod) and Hp 0. The assumed allele might be a "modified" form of Hp^2 (i.e. Hp^{2m}) or of Hp^1 (i.e. Hp^{1m}).

Neither the assumed modifier nor the assumed allele appears to have an effect on the genotypes $Hp^1\ Hp^1$ or $Hp^2\ Hp^2$, so far as the family data indicate. (The one possible exception, a Hp 0 child of a Hp 1-1 × Hp 1-1 mating, will be discussed later.)

In the case of a linked modifier, one would expect that in dihybrids the modifier would be on the same chromosome as the Hp allele it modifies as often as it would be on the homologous chromosome (Robbins, 1918). Obviously, a modifier on a chromosome not containing the Hp locus would segregate independently. Consequently, in either case, matings with one parent Hp 2-1 (mod) or Hp 0 and the other parent Hp 1-1, Hp 2-2 or Hp 2-1 would be expected to yield (among the heterozygous offspring) about equal numbers with and without the modifier if the modifier is rare. If it is frequent more than 50 per cent would have the modifier. Thus, one would expect the number of Hp 2-1 offspring to be approximately equal to the sum of Hp 2-1 (mod) plus Hp 0

TABLE 3. EXPECTED HAPTOGLOBIN GENOTYPES RESULTING FROM VARIOUS MATINGS, ASSUMING A THIRD ALLELE AT THE *Hp* LOCUS

(A) Assuming an allele, Hp^{1m}

Matings		Offspring	
Phenotype	Usual Genotype	Expected Genotypes	Ratio 2-1:2-1M + 0
1. 2-1 × 2-1M or 0	$Hp^2Hp^1 \times Hp^2Hp^{1m}$	$\frac{1}{4}Hp^2Hp^2$:$\frac{1}{4}Hp^2Hp^{1m}$ $\frac{1}{4}Hp^2Hp^1$:$\frac{1}{4}Hp^1Hp^{1m}$	1:1
2. 1-1 × 2-1M or 0	$Hp^1Hp^1 \times Hp^2Hp^{1m}$	$\frac{1}{2}Hp^1Hp^{1m}$:$\frac{1}{2}Hp^2Hp^1$	1:0*
3. 2-2 × 2-1M or 0	$Hp^2Hp^2 \times Hp^2Hp^{1m}$	$\frac{1}{2}Hp^2Hp^2$:$\frac{1}{2}Hp^2Hp^{m1}$	0:1†

(B) Assuming an allele, Hp^{2m}

1. 2-1 × 2-1M or 0	$Hp^2Hp^1 \times Hp^{2m}Hp^1$	$\frac{1}{4}Hp^2Hp^{2m}$:$\frac{1}{4}Hp^{2m}Hp^1$ $\frac{1}{4}Hp^2Hp^1$:$\frac{1}{4}Hp^1Hp^1$	1:1
2. 1-1 × 2-1M or 0	$Hp^1Hp^1 \times Hp^{2m}Hp^1$	$\frac{1}{2}Hp^1Hp^1$:$\frac{1}{2}Hp^{2m}Hp^1$	0:1†
3. 2-2 × 2-1M or 0	$Hp^2Hp^2 \times Hp^{2m}Hp^1$	$\frac{1}{2}Hp^2Hp^{2m}$:$\frac{1}{2}Hp^2Hp^1$	1:0*

* These ratios hold if the assumed allele is rare. If it is not, the ratio will be 1:X where X > 0 with its value determined by the frequency of the allele.

† These ratios hold regardless of the frequency of the assumed allele.

offspring. Hence approximately equal numbers of the two types would indicate a modifier, but would not distinguish between linkage and independence.

In considering the alternative hypothesis of a third allele at the Hp locus, note that the expected frequencies of the phenotype Hp 2-1 vs. the phenotypes Hp 2-1 (mod) and Hp 0 among the offspring of matings of Hp 2-1 by Hp 2-1 (mod) would be equal regardless of whether the allele is a modified form of Hp^1 or Hp^2 (Tables 3A and B, line 1). Hence, these matings are not useful for distinguishing among the alternatives of third alleles or a modifier. Only matings of Hp 1-1 or Hp 2-2 by Hp 2-1 (mod) or Hp 0 can help to distinguish among the alternatives.

If the allele is Hp^{1m}, matings between Hp 2-2 and Hp 2-1 (mod) or Hp 0 will yield no Hp 2-1 offspring. If the allele is Hp^{2m} matings between Hp 1-1 and Hp 2-1 (mod) will yield no Hp 2-1 offspring. Finally, if we are dealing with a modifier, each of these matings should yield approximately equal numbers of Hp 2-1 offspring and Hp 2-1 (mod) plus Hp 0 offspring. The various matings, their possible genotypes, and their outcomes are shown in Tables 3A and B.

The pertinent findings are summarized in Table 4. As predicted by all hypotheses, matings of Hp 2-1 × Hp 2-1 (mod) or Hp 0 yielded approximately equal numbers of Hp 2-1 and Hp 2-1 (mod) plus Hp 0 offspring (Table 4A).

There were no Hp 2-1 offspring and thirteen Hp 2-1 (mod) or Hp 0 offspring from matings of Hp 1-1 × Hp 2-1 (mod) or Hp 0 (Table 4B). This distribution is significantly different from a 1:1 ratio ($P < .0001$). As stated above, this is precisely what would be expected from an Hp^{2m} allele and the reverse of the prediction based on an Hp^{1m} allele.

Matings of Hp 2-2 × Hp 2-1 (mod) or Hp 0 yielded five Hp 2-1 and five Hp 0

offspring, but none with the phenotype Hp 2-1 (mod). If the allele is Hp^{2m} these data suggest that it is relatively common in this sample and that the three Hp 2-2 parents involved were genotypically $Hp^{2}\ Hp^{2m}$. The probability of such a sample will be computed in the next section, following a calculation of gene frequencies.

FREQUENCY OF THE HAPTOGLOBIN ALLELES

As a first approximation, the frequencies of the alleles Hp^{1} and $Hp^{2} + Hp^{2m}$ may be estimated from the parental data given in Table 5. Six parents were excluded from the calculation: one Hp 0 father with Hb SC, which could account for his phenotype (particularly since his offspring indicate he is Hp 2-1), and five because their parents were included in the sample. Then, assuming that all Hp 2-1 (mod) and Hp 0 parents are heterozygotes, the maximum likelihood estimate of the frequency of Hp^{1} is .539 $\pm$ 0.27.

The distribution of the remaining .461 alleles among Hp^{2} and Hp^{2m} can be calculated as follows:

The distinction between Hp 2-1 and the other two types is determined by whether Hp^{1} is associated with Hp^{2} or Hp^{2m}. These alleles must be associated with Hp^{1} in accordance with their relative frequencies in the population. Hp^{1} is associated with Hp^{2} or Hp^{2m} 57.3 per cent of the time (Table 5). It is associated with Hp^{2} 39.3 per cent of the time; hence, the frequency of Hp^{2} = (.393/.573) (.461) = .316, and the frequency of Hp^{2m} = .461 − .316 = .145.

The computed frequencies of the genotypes $Hp^{2}Hp^{2}$, $Hp^{2}Hp^{2m}$, and Hp^{2m} Hp^{2m} are shown in Table 6. Since the Hp^{2m} allele is present in .5331, (.4340 +

TABLE 4. NUMBER OF OFFSPRING WITH HAPTOGLOBIN PHENOTYPES 2-1, 2-1M AND 0 FROM MATINGS WITH ONE PARENT 2-1M OR 0

Mating	No.	Offspring		
		2-1	2-1M	0
A. 2-1 × 2-1M	10	9	7	6
2-1 × 0	3	3	0	2
Totals		12	15	
B. 1-1 × 2-1M	6	0	4	4
1-1 × 0	2	0	1	4
Totals		0	13	
C. 2-2 × 2-1M	2	5	0	3
2-2 × 0	1	0	0	2
Totals		5	5	

TABLE 5. DISTRIBUTION OF HAPTOGLOBIN PHENOTYPES AMONG THE PARENTS*

	Haptoglobin Phenotype					
	1-1	2-2	2-1	2-1M	0	Total
Number	45	31	70	27	5	178
Percent	25.3	17.4	39.3	15.2	2.8	100.0

* See text for reasons for excluding six parents

.0991) of Hp 2-2 individuals (ignoring the possibility that $Hp^2\ Hp^{2m}$ and $Hp^{2m}Hp^{2m}$ may occasionally be Hp 0), the probability that each of three Hp 2-2 individuals would carry at least one Hp^{2m} allele is $(.5331)^3$ or .1515. Hence, the assumption that each of the three Hp 2-2 parents in matings of Hp 2-2 × Hp 2-1 (mod) or Hp 0 (Tables 1 and 4c) carried at least one unexpressed Hp^{2m} allele is reasonable.

The three-allele hypothesis may be tested by comparing the distribution of the phenotypes among the parents and among the offspring on the assumption that the population is in Hardy-Weinberg equilibrium. The observed and expected frequencies derived by using the gene frequencies computed above are presented in Table 7. The distributions do not differ significantly from the expected values.

Comparison of the phenotype frequencies among the parents and children (Table 7) indicates that ahaptoglobinemia (Hp 0) is less frequent, while Hp 2-1 (mod) is more frequent among the parents thad among their offspring, ($\chi^2_{(1)} = 10.64$; $P < .01$, and $\chi^2_{(1)} = 7.27$; $P < .01$, respectively). The combined frequency of Hp 0 and Hp 2-1 (mod) in the parents is not significantly different from that

TABLE 6. EXPECTED FREQUENCIES OF THE GENOTYPES Hp^2Hp^2, Hp^2Hp^{2m}, AND $Hp^{2m}Hp^{2m}$

Genotype	Frequency	
	Among the Total Population	Among the Phenotype, Hp 2-2
Hp^2Hp^2	.099	.4669
Hp^2Hp^{2m}	.092	.4340
$Hp^{2m}Hp^{2m}$	.021	.0991
	.212	1.0000

TABLE 7. OBSERVED AND EXPECTED FREQUENCIES OF THE HAPTOGLOBIN PHENOTYPES AMONG THE PARENTS AND OFFSPRING*

A. Parents

	1-1	2-2	2-1	2-1M	0	Total
				27	5	
Observed	45	31	70	32		178
Expected	51.6	37.9	60.7	27.8		178

$\chi^2_{(1)} = 4.16$; $P \sim .04$

B. Offspring

	1-1	2-2	2-1	2-1M	0	Total
				23	38	
Observed	98	56	92	61		307
Expected	89.2	65.2	104.6	48.0		307

$\chi^2_{(3)} = 7.23$; $P > .05$

* The expected frequencies were computed assuming a Hardy-Weinberg equilibrium, using gene frequencies derived from the parental population.

in the children ($\chi^2_{(1)} = 0.25$; $P > .5$). This suggests that the Hp 0 phenotype may change to Hp 2-1 (mod) as an individual ages. Such an assumption is consistent with the finding of Galatius-Jensen (1958b) that decreased haptoglobin production occurs more commonly in children than in adults.

DISCUSSION

The analysis suggests that the phenotype Hp 2-1 (mod) is always, and Hp 0 nearly always genotypically $Hp^{2m}Hp^1$. If this is correct, the Hp 0 child of a Hp 1-1 × Hp 1-1 mating (Table 1) is probably extramarital.

The data presented here are not sufficient to determine the phenotype of Hp^{2m} homozygotes with certainty. One child from a mating of Hp 2-1 (mod) × Hp 2-1 (mod) had a small amount of haptoglobin which appeared to be Hp 2-2. This indicates that at least sometimes, the genotype $Hp^{2m}Hp^{2m}$ can yield the Hp 2-2 phenotype.

On the basis of the three allele theory, matings of Hp 2-1 (mod) × Hp 2-1 (mod) would be expected to yield offspring with the distribution ¼ Hp 1-1:¼ Hp 2-2:½ Hp 2-1 (mod) + Hp 0, but no Hp 2-1. As shown in Table 1, these expectations are fulfilled ($\chi^2_{(2)} = 5.2351$; $P > .05$), although there is a deficit of homozygotes, particularly Hp 2-2. A similar deficit of Hp 2-2 offspring appears in matings of Hp 2-1 × Hp 2-1 (mod). Again, the difference between observed and expected is not significant. This deficit does not appear in the Hp 2-1 × Hp 2-1 matings. On the contrary, there is a slight excess of Hp 2-2 offspring. In all instances, the sample is too small to permit a decision concerning the validity of the differences. However, the possibility exists that the Hp 2-2 deficit may be due to the fact that both the genotypes $Hp^{2m}Hp^{2m}$ and Hp^2Hp^{2m} can be phenotypically Hp 0, particularly in children. This assumption receives some support from the data of Nyman (1958) who reported that the quantity of haptoglobin in the serum of individuals with Hp 2-2 is usually less than that present in the serum of Hp 1-1 and Hp 2-1 Caucasian subjects.

Tentatively, the data presented here may be explained by assuming a third allele, Hp^{2m}, at the Hp locus which, when heterozygous with Hp^1, usually yields the phenotypes Hp 2-1 (mod) and Hp 0. Ahaptoglobinemia (i.e. Hp 0) may also result from the genotype Hp^2Hp^1, if $Hp^{2m}Hp^1$ never yields the phenotype Hp 2-1 (see the matings of Hp 1-1 × Hp 2-1 and of Hp 2-1 × Hp 2-1 shown in Table 1). The additional possibility that the genotypes Hp^2Hp^{2m} and $Hp^{2m}Hp^{2m}$ are occasionally represented by the Hp 0 phenotype cannot be excluded on the basis of the sample. There is no evidence in the present data to indicate that Hp^1Hp^1 or Hp^2Hp^2 are ever Hp 0. Resolution of the problem of ahaptoglobinemia inheritance requires an extension of this investigation to include a much larger number of families.

SUMMARY

An investigation of the inheritance of the haptoglobin phenotypes was carried out by performing starch gel electrophoresis on the plasma of 492 members of 92 Negro families. Analysis of the results indicates that in addition to the usual

alleles, Hp^1 and Hp^2, a third allele, Hp^{2m}, is present in these subjects, its frequency being approximately .145. This allele, in combination with Hp^1, produces either the phenotype Hp 2-1 (mod) or Hp 0 (ahaptoglobinemia) and possibly Hp 2-1.

That ahaptoglobinemia (not due to excessive red cell destruction) may also be the result of the genotype Hp^2Hp^1 is possible from the data. However, there is only suggestive evidence that Hp 0 may also be found in individuals with the genotypes Hp^2Hp^{2m} and $Hp^{2m}Hp^{2m}$. The presented data gave no indication that the homozygotes, Hp^1Hp^1 and Hp^2Hp^2 are ever phenotypically Hp 0, but this possibility cannot be entirely ruled out on the basis of a sample of this size.

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Survival Trends in a Senescent Twin Population

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DESPITE the time-honored maxim that "it matters not how long you live, but how well", man goes right on striving to live both long and well. With an average life span of nearly 70 years already achieved in our time, it begins to look as if our youngest generations may very well succeed in realizing this dream. At any rate, as current life expectancies slowly approach certain limits set by genetically determined longevity potentials, the need becomes greater for a better understanding of the biological aging phenomena responsible for variable impediments of health in the later stages of the life cycle.

Adequate data on the contribution of genic elements to the scheme of human health and survival are as scarce for the gerontological period as for the other stages of the life span. However, evidence for the significant part played by genetic factors in determining life span potentials has been obtained from a number of sources. The average duration of life varies greatly throughout the animal kingdom, from four days for the housespider to 177 years for the Galapagos tortoise (Spector, 1956) and this remarkable variability cannot be explained on other than a gene-specific basis. The same assumption may be made about the well-known life span difference between the sexes. With the female in all species studied so far tending to outlive the male, the theory that the way of life adopted by man is less stressful for the female than the male appears clearly insufficient to account for the total sex difference in longevity.

Additional support for the genetic control of life span potentials has been derived from family data, comparing filial and parental age variations, as well as from twin studies. Surveys in different countries indicated that children of long-lived parents tend to live longer than those of short-lived parents (Pearl and Pearl, 1934; Jalavisto, 1951; Kallmann, Aschner and Falek, 1956; Kallmann and Jarvik, 1959), and this general trend was confirmed by the preliminary statistics accumulated in our own twin sibship study which was organized in New York State in 1945 (Kallmann and Sander, 1948, 1949; Feingold, 1950).

The present report summarizes some of our final life span data collected during a 12-year follow-up period from a total of 1603 white twin index cases who survived to age 60. Table 1 illustrates the sources from which this sample was gathered. As expected, females exceeded males by a considerable margin (910 and 693)

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TABLE 1. SOURCES AND SEX DISTRIBUTION OF 1603 WHITE SENESCENT TWIN INDEX CASES IN NEW YORK STATE

	General Population	Homes for the Aged	Mental Hospitals	Other Hospitals	Total
Male	456	51	167	19	693
Female	597	65	241	7	910
Total	1053	116	408	26	1603

TABLE 2. ZYGOSITY OF 1019 PAIRS FORMED BY 1603 SENESCENT TWIN INDEX CASES

	Monozygotic	Same-Sex Dizygotic	Opposite Sex	Unclassified	Total
Male	116	114	—	74*	304
Female	145	183	—	92**	420
Total	261	297	295	166	1019

* Includes 4 pairs where cotwin's sex unknown
** Includes 6 pairs where cotwin's sex unknown

among the index cases from institutions as well as in the group originally culled from the general population (56.9 and 56.7 %, respectively). The overall ratio of 100 females to 76 males (56.8 % females) was slightly higher than that (100:89) for the corresponding total white population of New York State (Table 15 of the 1950 census). This excess seems to be accounted for, in part at least, by one-egg females concordant for survival past the age of 60.

It was also according to expectation that the largest proportion of index cases (1053 or 65.7 %) came from the general population. A certain deficit of twins among residents in homes for the aged (116 or 7.2 %) as compared with mental hospital patients (408 or 25.4 %) was probably due to the fact that ascertainment depended upon the inmates themselves, many of whom were in advanced stages of senility and had no surviving relatives. By comparison, the ascertaining of twins among mental hospital patients was virtually complete, largely because of the availability of detailed family histories.

The zygosity classification of the 1019 pairs, formed by our sample of 1603 senescent twin index cases, is presented in Table 2. In 584 pairs, both twins qualified as index cases, while the remaining 435 pairs contributed only one index case per pair—the cotwins having either died before the age of 60 or failed to fulfill our residency requirement.

If the 166 unclassified pairs are disregarded (mostly with a cotwin of the same sex who died at an early age), the ratio of one-egg to two-egg pairs in our sample is 261:592. With a rate of 30.6 per cent for one-egg pairs, our figure falls between the percentages of 25.6 and 32.6 compiled by Weinberg (1902) and Yerushalmy and Sheerar (1940), respectively, for the proportion of monozygotic twins born in the United States (1890–1900) and the State of New York (1936–1937). Of course, these percentages have come from birth statistics, while no adequate estimate is available of the proportions of one-egg and two-egg twins expected in the age group 60 and over (Allen, 1955). However, the representativeness of our senescent

sample is reflected by the two-egg group, where the ratio of same-sex to opposite-sex pairs (297:295) equals the theoretically expected 1:1 ratio.

The preponderance of female pairs in the same-sex group (420 out of 724, or 58%) is an expression of their longer life span. That the deficit of males in this sample is due to their abbreviated longevity rather than to some sampling bias is borne out by the life span data provided by our series of 753 deceased index twins. The mean life span of 348 males was approximately three years shorter than that of 405 females (891 and 930 months, respectively).

The current survival status of our original series of 1603 senescent *twin index pairs* is reviewed in Table 3. (A twin index pair consists of the index case and his cotwin. If two index cases are members of the same twin pair, they form two separate twin index pairs.) Despite our long observation period, one or both members in 1055 index pairs were still alive, either at the time of our last communication with them, or on January 1, 1958, the closing date for this analysis. Thus the life histories of about two-thirds of the index pairs (65.8%) remained incomplete for our final analysis. (The corresponding proportion for the 1019 twin pairs formed by these 1603 index cases is 67.3%—see Appendix.) The largest contributions to these "broken" and surviving pairs were made by the opposite-sex and unclassified groups (76.8 and 76.6%, respectively), since they were the very groups for which no systematic follow-up could be undertaken. In some of these cases, the first contact was also the last.

Regardless of zygosity, among like-sexed pairs for which recent information is available, a smaller proportion of male pairs (15.4% MZ and 15.5% DZ) than female pairs (33.6% MZ and 27.5% DZ) had both members living. This difference may be ascribed to the shorter life span of the male and is also reflected in the category of "both dead", which shows a slightly higher percentage of male than of female index pairs, of the one-egg (42.3 and 35.5%, respectively) as well as of the two-egg variety (41.2 and 35.7%, respectively).

The proportions of one-egg and same-sexed two-egg index pairs in the various

TABLE 3. CURRENT SURVIVAL STATUS OF 1603 SENESCENT TWIN INDEX PAIRS (INDEX CASES AND THEIR COTWINS)

		One Dead		Both Dead		
	Both Alive	Died <60 yrs	Died ≧60 yrs	One Died <60 yrs	Both Died ≧60 yrs	Total
One-Egg Male	32	6	78	4	88	208
Two-Egg Male	30	19	61	4	80	194
One-Egg Female	89	8	66	8	94	265
Two-Egg Female	84	27	71	14	109	305
Opposite-Sex	152	88	87	32	67	426
Unclassified	39	84	34	23	25	205
All Male	151	93	196	40	213	693
All Female	275	139	201	45	250	910
All One-Egg	121	14	144	12	182	473
All Same-Sex Two-Egg	114	46	132	18	189	499
Total	426	232	397	85	463	1603

survival categories correspond closely. The percentage of MZ pairs falling into the category of "both alive" (25.6%) is moderately higher than that of same-sex dizygotic pairs (22.8%). Conversely, fewer one-egg pairs appear in the group of index pairs broken by the death of one twin, particularly in that subgroup where the cotwin died below the age of 60 (3.0 and 9.2%, respectively). This finding may be taken as an indication of the tendency of one-egg twins to correspond more closely than two-egg twins in life spans.

To evaluate more directly the observed intra-pair similarities in life span for the different sex and zygosity groups, successive biennial analyses were made as soon as same-sex pairs with two deceased partners became available. The results of these earlier comparisons are summarized in Table 4, together with the final data. Only those pairs have been included in which both partners died after the age of 60 of verified natural causes. The preliminary entries for 1948 and 1952 were expressed in terms of twin index pairs (Kallmann, Aschner and Falek, 1956), while the remaining computations were made in relation to the total number of twin pairs in each age group, regardless of index status. Another factor which reduced the number of pairs included in this analysis was the requirement that the cause of death given on the death certificate had to be adequately confirmed. Hence, any pertinent suspicion of the naturalness of one twin's death was sufficient to eliminate the pair from subsequent analyses (Kallmann and Jarvik, 1959).

The intra-pair differences in life span have been consistently greater for two-egg than for one-egg pairs (Table 4). This difference has been particularly pronounced in the female series, where the mean life span differences of two-egg twin

TABLE 4. SUCCESSIVE BIENNIAL ANALYSES OF MEAN INTRA-PAIR DIFFERENCES IN LIFE SPANS OF SAME-SEX SENESCENT TWIN PAIRS OVER AGE 60

Type of Twins	Year of Analysis	Number of Pairs	Intra-Pair Life Span Differences (In Months)		
			Male	Female	Total
One-Egg Pairs	1948[1]	32	47.6	29.4	36.9
	1950	11	48.0	18.2	34.4
	1952[1]	76	40.7[2]	30.7[2]	35.7[2]
	1954	24	50.6	22.9[2]	37.9[3]
	1956/7	41	60.0	36.9[3]	48.7
	1958/9 (Final)	75	62.5	55.4	58.8
Two-Egg Same-Sex Pairs	1948[1]	36	89.1	61.3	78.3
	1950	17	73.8	41.3	68.1
	1952[1]	86	79.1[2]	69.5[2]	73.7[2]
	1954	37	68.9	65.6[2]	67.0[3]
	1956/7	45	64.5	68.2[3]	66.5
	1958/9 (Final)	88	65.0	80.9	74.4[4]

[1] The analyses made in 1948 and 1952 were expressed in terms of twin index pairs and were preliminary in scope.

[2] Difference between one-egg and two-egg pairs significant at 1% level.

[3] Difference between one-egg and two-egg pairs significant at 5% level.

[4] Mean intra-pair life span difference for opposite-sex pairs: 102.2 months (final analysis).

partners have exceeded those of one-egg twins by at least two years, reaching statistical significance on three occasions.

Although one-egg twins tend to be more alike in their life spans than two-egg twins, the extent of this similarity diminishes with the progression of the observational period. Initially, members of one-egg pairs differed in their average life span by about three years, while the corresponding difference in two-egg pairs was over six years. By the time of our last analysis, the mean difference in ages at death had risen to almost five years in the one-egg group (58.8 months), with no corresponding change in the two-egg group (74.4 months). The gradual merging of the two zygosity groups is particularly noticeable in the male series, where the given difference between monozygotic and dizygotic groups dwindled to two and a half months (62.5 and 65.0 months, respectively). Because of the very nature of a longitudinal study, extreme intra-pair differences in survival apparently become manifest only as the observation period is extended, thus accounting for the increase in life-span differences of one-egg pairs (Kallmann and Jarvik, 1959).

Another explanation would be that increasing uniformity has to be expected as more and more twins approach the asymptotic portion of the survival curve. In addition, it may be assumed that two-egg twins will show increasing biological homogeneity with advancing age, since any two individuals who have survived together to a very old age at a given time must have been selected for superior health and survival values. Because of marked differences in life spans of the two sexes, this effect was expected to express itself earlier in male than in female pairs. It was anticipated, therefore, that in the male series differences between the two zygosity groups would no longer be detectable at an age where intra-pair life span differences between one-egg and two-egg female twins had only just begun to diminish.

It is possible to test our working hypothesis, and the data are presented graphically in Figure 1. In constructing the graph, the 163 same-sex pairs where both partners died over age 60 of verified natural causes have been arranged according to the age group of the first-deceased twin, with mean intra-pair life span differences plotted for three separate age groups (60–69, 70–79, 80 and over). It is apparent that once dizygotic twin brothers have survived together to the age of 70, their ultimate mean difference in life span may be expected to be of the same order of magnitude as that of monozygotic twins of either sex. Yet it will be noted that the mean intra-pair difference in the life spans of two-egg twin sisters who survived into their seventies still exceeds that of one-egg twin sisters by almost two years (79 and 57 months, respectively).

This difference was even more pronounced when the first twin sister died at a younger age. We had only nine monozygotic and nine dizygotic female pairs of this kind in the age group 60–69. In the one-egg series, the female twins died less than seven years after their cotwins (81 months), while the mean difference in survival time for the two-egg twins was more than twice as long (173 months). This difference between the two female zygosity groups is statistically significant at the five per cent level of confidence. Even in the oldest group, where both twins survived at least to age 80, the mean intra-pair difference in the life spans of two-

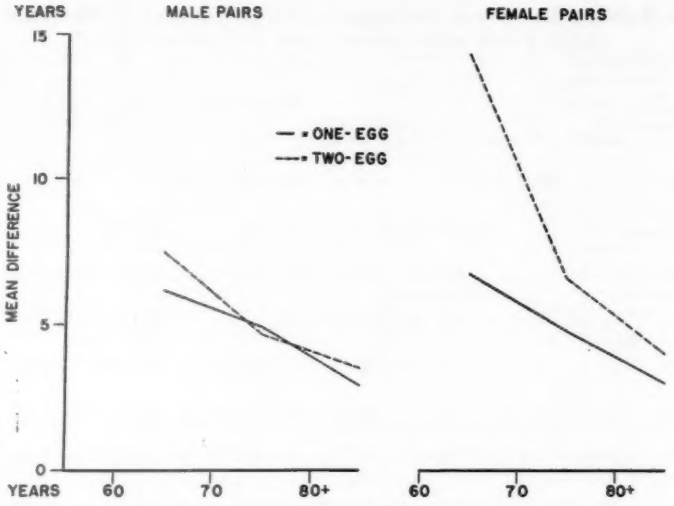

FIG. 1. Mean differences in longevity.

egg twin sisters still amounted to four years, in contrast to three years for the one-egg pairs.

The data used in Figure 1 were limited to three age groups and same-sex pairs. The data are presented in greater detail in Table 5. The first three age groups (15–59 years) have been included for completeness, although they are not strictly comparable to the remaining five age groups. The added groups contain those twin pairs in which one member died *before* age 60.

The structure of our study imposed a minimum intra-pair life span difference upon this group, inasmuch as one of the criteria for acceptance as index case stipulated survival of one twin partner beyond age 60. By definition, therefore, the intra-pair difference in life span had to equal *at least* the difference between the age at death of the first-deceased twin and age 60. For example, if one member of a given pair died at age 50, the minimum possible intra-pair life span difference was ten years. Any difference smaller than that excluded the pair from our study, since both twins would have failed to survive until age 60. In spite of this artefact which applied equally to all pairs, regardless of sex or zygosity, intra-pair differences have always been larger for two-egg than for one-egg pairs.

In both sexes, in the age groups above 60 years the mean intra-pair life span differences of two-egg pairs consistently exceed those of one-egg pairs. In a series of ten such comparisons all 10 would be expected to be in the same direction by chance less than once in a thousand times ($1/2^{10}$). It is, therefore, justifiable to conclude that gene-specific elements play an important role in determining a wide range of variations in man's natural life span.

In view of the finding that various factors operate toward a state of biological uniformity in any group approaching the upper limit of man's life expectancy, it

TABLE 5. MEAN INTRA-PAIR DIFFERENCES IN LIFE SPAN OF 235 TWIN PAIRS WHERE BOTH PARTNERS DIED OF NATURAL CAUSES*

Age Group First Deceased (In Years)	Male		Female		Opposite Sex	Unclassified	All One-Egg	All Same-Sex Two-Egg	All Same-Sex Pairs	
	One-Egg	Two-Egg	One-Egg	Two-Egg					Male	Female
15–49	—	388 (2)**	—	544 (4)	390 (6)	431 (3)	—	492 (6)	399 (3)	509 (6)
50–54	—	—	230 (1)	—	160 (2)	—	230 (1)	—	—	230 (1)
55–59	201 (3)	—	135 (6)	215 (4)	242 (1)	—	157 (9)	215 (4)	201 (3)	167 (10)
60–64	113 (7)	130 (2)	102 (3)	215 (5)	138 (8)	134 (1)	110 (10)	190 (7)	119 (10)	172 (8)
65–69	39 (8)	82 (11)	71 (6)	122 (4)	183 (3)	70 (4)	53 (14)	93 (15)	68 (22)	83 (11)
70–74	66 (13)	78 (6)	68 (7)	97 (6)	80 (7)	115 (3)	66 (20)	87 (12)	72 (20)	85 (15)
75–79	38 (4)	42 (9)	49 (9)	70 (13)	68 (5)	65 (2)	46 (13)	59 (22)	39 (14)	64 (23)
80+	35 (4)	42 (8)	36 (14)	48 (24)	68 (6)	2 (1)	36 (18)	46 (32)	39 (12)	42 (39)

* 8 monozygotic and 3 dizygotic pairs excluded because of some question concerning zygosity. 3 opposite sex and 2 unclassified pairs where one partner died below 15 years also excluded.

** Figures in parentheses refer to number of twin pairs.

is rather remarkable that in our sample the vigorous expression of genetic survival potentials has not yet been wiped out. The declining trend in intra-pair life span differences is manifested in all groups, regardless of sex or zygosity (Table 5).

Another lesson conveyed by these data (Table 5) may be helpful for the organization of similar twin studies of aging in the future. With the benefit conferred by hindsight it is now possible to say that the initial age limit should be set lower for male than for female twins when the total range of aging is to be observed. Apparently, the age of 60 was a good choice for our sample of female twins, but an initial age level of 50 or 55 years might have been more realistic for male pairs.

It would also be desirable to enlarge the quantitative scope of such a study. We could not have foreseen that after 12 years of observation, a series of 1019 pairs formed by our sample of 1603 white twin index cases would shrink, when a complete life history was set as a prerequisite for both partners, to two dizygotic male pairs with the first-deceased twin in the age group 60–65. Since pairs with complete histories had to be divided into five categories, according to sex and zygosity, for each of the five age groups beyond the age of 60, making a total of 25 subgroups, it may be argued in retrospect that 5000 rather than 1000 aging twin pairs would have given us a more effective baseline for our final analysis. The fact remains, however, that the total population to be screened for such a sample would have to be five times as large as the white population of New York State, or about 70 million people.

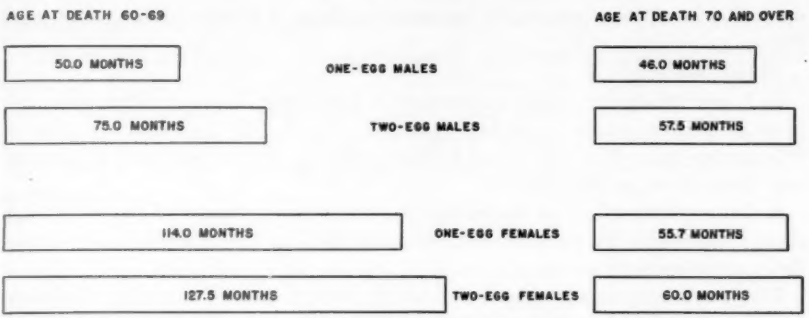

FIG. 2. Current median survival time (in months) of living twins of pairs broken by the death of one partner.

To overcome some of the statistical disadvantages imposed by the small number of pairs in certain subgroups, an attempt has been made to utilize the life span data of broken pairs (one twin deceased, the other alive at the time of our last inquiry) in our analysis of comparative longevity potentials. Official life expectancy values based on life tables for white males and females in the United States (U. S. Life Tables, 1954*) have been added to the last-known ages of surviving cotwins, although this procedure would inevitably dilute the expression of genetically determined inter-group differences. Apart from the fact that the same life expectancies had to be given to surviving members of one-egg and two-egg pairs, without regard to any expected zygosity differences, our system required that only those broken pairs could have been considered where a natural cause of death had been established for the first-deceased twin.

This combination of actual intra-pair differences for completed pairs (Table 5) and expected intra-pair differences for broken pairs (difference between expected age of survival and actual age of death in the cotwin) led to the desired increase in the number of pairs for each of the 25 subgroups. The new totals are 359 classified pairs (instead of 192) and 288 same-sex pairs (instead of 163). Interestingly, the new differential values for two-egg pairs, although considerably reduced in magnitude, still exceed those for one-egg pairs, except for male pairs in the age groups over 75. The figures for male pairs are 103 and 98 months, respectively, and for female pairs 138 and 115 months, respectively (all age groups over 60).

To avoid artificial dilution of inter-group life span differences, it seemed preferable to evaluate broken and completed pairs separately, as has been done in Figure 2 for 117 same-sex broken pairs according to the ages at death of the deceased twin partners. (Eight pairs were excluded because the last contact with the living twin preceded the death of the cotwin.) The intra-pair differences represent only *minimum values*, since the cotwins are known to have had a median survival time of at least the given number of months. Nevertheless, the broken

* For those cotwins who were listed as "missing" at various earlier dates, appropriate corrections were based on 1901 or 1910 Life Tables.

pairs (Figure 2) show essentially the same trends as the series of completed pairs (Table 5).

These trends are:

a. A general decline in the magnitude of intra-pair life span differences with increasing age at death of the first-deceased twins. In the highest age ranges, intra-pair life span differences tend to reach similarly low levels in all subgroups, regardless of sex or zygosity.

b. Despite this effect of increasing biological homogeneity in the oldest age groups, intra-pair life span differences in the two-egg group consistently exceed those in the one-egg group.

The general conclusions to be drawn at the present stage of our analysis of comparative life span data, obtained in a 12-year follow-up study of aging twins in New York State are:

1. The decision to bring to a close our statewide, longitudinal study of senescent twins after 12 years of observation has not been the natural consequence of dealing with a population that survived the first six decades of life. Instead, with 53 per cent of the original series of 1603 white twin index cases still alive at the time of our last contact, an arbitrary final date had to be set, since the likelihood of being able to follow the histories of all twins until the completion of their natural life spans had become rather remote.

2. Analysis of the data on the relation between longevity variations and differences in sex and zygosity has shown that male twin index cases, regardless of zygosity, tend to have a shorter life span than female twins. The mean length of life of 348 deceased males has been approximately three years shorter than that of 405 deceased females (891 and 930 months, respectively).

3. Mean intra-pair differences in life span have been found to be consistently higher in two-egg than in one-egg pairs, particularly in the female series. The difference between the two zygosity groups was most pronounced in the younger age groups (first-deceased twin died between the ages of 60 and 70) and gradually disappeared in the oldest age group (80 and over), especially in the male series. This is in accordance with the hypothesis that members of any group of aging persons are apt to show increasing homogeneity as they approach the upper limits of their natural life span, because they have been selected for superior health and survival values at some point.

Because of their generally shorter life span, males tend to manifest this effect several years earlier than females. Eventually, the mean intra-pair life span differences of two-egg pairs reach the low level characteristic of one-egg twins.

4. Evaluation of the known survival time of living twin partners in pairs broken by the death of one twin confirms the differential trends in intra-pair life span variations observed in pairs with two deceased twin partners.

5. Finally, it may be said that the importance of genetic elements in the determination of man's life span has been fully demonstrated. Therefore, in any proper evaluation of the variables affecting man's total length of life, genetic differences have to be taken into account.

ACKNOWLEDGMENTS

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APPENDIX. CURRENT SURVIVAL STATUS OF 1019 SENESCENT TWIN PAIRS

	Both Alive	One Dead		Both Dead		All Pairs
		Died Below 60	Died at 60 or over	One Died Below 60	Both Died Over 60	
Monozygotic Male	18	6	41	4	47	116
Dizygotic Male	17	19	33	4	41	114
Monozygotic Female	46	8	34	8	49	145
Dizygotic Female	46	27	37	14	59	183
Opposite Sex	81	88	56	32	38	295
Unclassified	24	84	21	23	14	166
All Male	45	60	82	22	95	304
All Female	106	84	84	31	115	420
All Monozygotic	64	14	75	12	96	261
All Dizygotic Same Sex	63	46	70	18	100	297
All Pairs	232	232	222	85	248	1019

ABO-Rh Interaction in an Rh-Incompatibly Mated Population*

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THE INVESTIGATION of the possible deleterious effects of blood group incompatibility between mother and fetus dates back almost to the discovery of the ABO blood groups themselves (Dienst, 1905; von Dungern and Hirszfeld, 1909). Yet certain aspects of the problem—the biological mechanism of selection, the interaction of different selection pressures, and the impact of these pressures on allele frequencies in succeeding generations—still remain a challenge today.

Even more interesting than the effects of incompatibility at single blood group loci are the effects of the *dual incompatibilities*, ABO and Rh. As customarily defined, *Rh-incompatibility* is the situation where the fetus is Rh positive (Rh_0 or D positive) and the mother is Rh-negative (really Rh_0 or D negative); and *ABO-incompatibility*, where the fetus carries an A or B antigen lacking in the mother.

The keen observation by Levine (1943) that the Rh-negative mothers of erythroblastotic offspring are less frequently ABO-incompatibly mated than expected has been confirmed by numerous investigations in which a sample of Rh-sensitized mothers and their families has been compared to random expectancy or control groups, mainly or partly Rh-compatibly mated (Wiener, 1945; Race, Taylor, Cappell and MacFarlane, 1943; Broman, 1944; van Loghem and Spaander, 1948; Speiser and Jancik 1953; as well as Malone, 1949; Lucia and Hunt, 1950 a, b, c; Brendemoen, 1952; Wiener, Nappi, and Gordon, 1953; Heistö, 1955; Nevanlinna, 1953; Nevanlinna and Vainio, 1956; Reepmaker, 1956). While such studies yield evidence consistent with the hypothesis of a protective action of ABO-incompatibility against Rh-sensitization, they do not exclude other explanations. This investigation was therefore undertaken to clarify the interplay of the two incompatibilities by utilizing a *nonsensitized* Rh-incompatibly mated series as a control for the *sensitized* Rh-incompatibly mated series—a different approach from other investigations.

The classification of population samples on the basis of Rh mating status is given in Chart 1.

The ABO-compatible, ABO-incompatible, and potentially ABO-incompatible combinations are listed in Chart 2. The percentages of ABO-incompatible offspring expected from various population segments (mothers, fathers, offspring) are given in Table 1.

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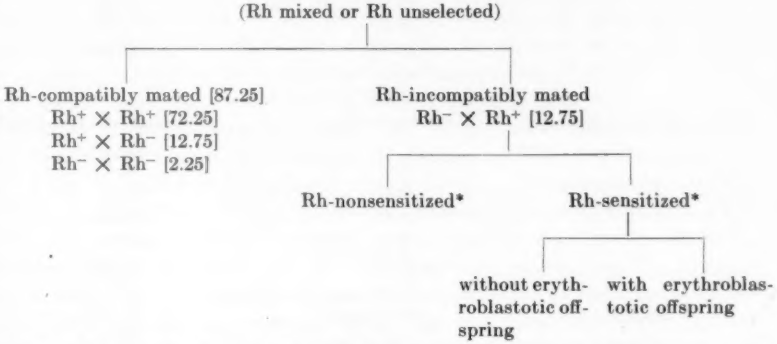
CHART 1. Classification of Population Samples on the Basis of Rh Mating Status



Rh mixed or unselected samples: L. and H. Hirszfeld and coworkers (1925, 1928); Levine (1943); Waterhouse and Hogben (1947); Johnstone (1954a); Boorman and coworkers (1945, 1949); Bryce, Jakobowicz, McArthur and Penrose (1950); Kirk, Kirk and Stenhouse (1953); Kirk, Shield, Stenhouse and Jakobowicz (1955); Reed and Kelly, (1958).

$Rh^+ \times Rh^+$ (primarily because of the low frequency of Rh^- in the Japanese population): Matsunaga (1955, 1956, 1958, 1959).

$Rh^- \times Rh^-$ and $Rh^- \times Rh^+$: Bresler (presented at 1959 meetings American Society of Human Genetics)

Rh-sensitized ($Rh^- \times Rh^+$): Malone, 1949; Lucia and Hunt, 1950; Brendemoen, 1952; Wiener, Nappi, and Gordon, 1953; Heistö, 1955; Nevanlinna and Vainio, 1956; Reepmaker, 1956.

Rh-sensitized (with erythroblastotic offspring): Levine, 1943; Wiener, 1945; van Loghem and Spaander, 1948; Speiser and Jancik, 1953.

* Rh-nonsensitized and Rh-sensitized ($Rh^- \times Rh^+$): This study.

MATERIAL

Source and Ascertainment

The group studied here is taken from the records of the Baltimore Rh Typing Laboratory where cards of Rh-negative nonsensitized women and their pregnancy histories (the Rh NS series) are filed separately from those of the Rh-sensitized women (the Rh S series). The Rh-nonsensitized series (Rh NS), derived from the laboratory records for the years 1945 through July, 1954, consists of families of Rh-negative nonsensitized women whose husbands are known to be Rh-positive (with the exception of a few mother-offspring combinations included in which the paternal blood type was unknown). The Rh-sensitized series (Rh S), derived from the records for the years 1945 through July 1955, inclusive, consists of families similarly Rh-incompatibly mated but in which, in addition, the mothers have been shown to possess Rh antibodies.

It is to be noted that sizes of Rh NS and Rh S series are *not* in proportion to one another as in the population, because ascertainment is clearly not equal in the two series. Where Rh immunization or pregnancy difficulties are anticipated,

CHART 2. ABO-Incompatible and ABO-Compatible Combinations

Mother-Offspring Combinations:	ABO-Compatible	ABO-Incompatible
	O-O	O-A
	A-O	O-B
	A-A	A-B
	B-O	A-AB
	B-B	B-A
	AB-A	B-AB
	AB-B	
	AB-AB	
Father-Offspring Combinations:	**ABO-Compatible**	**Potentially ABO-Incompatible**
	O-O	A-A
	O-A	A-AB
	O-B	B-B
	A-O	B-AB
	A-B	AB-A
	B-O	AB-B
	B-A	AB-AB
Matings:	**ABO-Compatible**	**Potentially ABO-Incompatible**
	O × O	O × A
	A × A	O × B
	B × B	O × AB
	AB × AB	A × B
	A × O	B × A
	B × O	A × AB
	AB × O	B × AB
	AB × A	
	AB × B	

Note: The father-offspring and mating combinations are designated as "potentially" ABO-incompatible since the same combination may be involved in either an ABO-compatible or an ABO-incompatible situation, e.g., A father-A offspring is ABO-compatible if the mother is A or AB but ABO-incompatible if the mother is O or B.

TABLE 1. PERCENTAGE OF ABO-INCOMPATIBLE OFFSPRING EXPECTED* IN THE Rh NS AND Rh S SERIES

Population Segment	Expected Percentage of ABO-Incompatible Offspring	
	Rh NS Series	Rh S Series
Mothers		
O	32.50	32.48
B	24.15	24.08
A	8.17	8.23
AB	0	0
Fathers		
O	0	0
A	33.00	33.05
B	44.25	44.21
AB	70.85	70.86
Offspring		
O	0	0
A	32.06	32.12
B	43.01	42.97
AB	80.59	80.60

* Expected based on the Hardy-Weinberg equilibrium and ABO allele frequencies for U. S. Whites and Negroes (Glass and Li, 1953). Because the racial composition was slightly different in the matings recorded for the Rh NS and Rh S series, the expected values are not identical.

both the attending obstetrician and the family are more cooperative in regard to laboratory and case history follow-up studies. Accordingly, no attempt will be made to combine the Rh NS and Rh S series; instead each series will be considered separately and in comparison with other series. It is reasonable to assume, however, that in neither series was cooperation by the physician or patient biased by ABO blood group constitution, even if it was known.

The terms "multiple ascertainment" and "single ascertainment" refer to parent-offspring combinations. Multiple ascertainment indicates that each offspring of known blood group and sex is entered in a separate parent-offspring combination. Thus if one mother has several children of known blood group and sex, the one mother is included several times, once in each mother-offspring combination. Single ascertainment indicates that one mother can be included in only a single mother-offspring combination, irrespective of the number of her offspring. In single ascertainment the oldest child of known blood group and sex has been used to represent the family in mother-offspring combinations.

Size of Samples

When multiple ascertainment is used, there are 3577 mother-offspring combinations in the Rh NS series and 2998 in the Rh S series, representing 3037 and 1335 individual families, respectively.

The Rh NS series includes 2313 families with 5064 births. The births include:

2887 offspring of known ABO blood group
4842 offspring of known sex
2850 offspring of known ABO blood group and sex

The Rh S series includes 1383 families with 4160 births. These births include:

2778 offspring of known ABO blood group
3678 offspring of known sex
2631 offspring of known ABO blood group and sex

The above categories are not mutually exclusive.

Race

Mixed racial groups are contained in both the Rh NS series and the Rh S series. Only white and Negro classifications were available. In the Rh NS series, 86.77 percent of the mothers are White, whereas in the Rh S series 90.20 percent are white (based on mothers in appendix Table I). This slight difference is reversed when matings only are considered, the Rh NS series then yielding 93.27 percent whites and the Rh S series 92.18 percent whites. This reversal suggests that the variation is not of biological significance but rather a function of socio-economic factors.

In computing the Hardy-Weinberg expectancies, individuals of unknown race were apportioned according to the proportion of races observed in the same series.

Maternal Ages

The mean ages shown in appendix Table IV reveal no significant differences among O, A, B, and AB mothers, within either series. Moreover, the distribution

of maternal ages (grouped in 5-year categories) analyzed by the mothers' ABO blood groups or by mating category showed no significant differences *within* either series.

A comparison *between* series, however, indicates a significantly greater mean age (28.625 ± .149) for the mothers of the Rh S series than for the mothers of the Rh NS series (27.237 ± .106). Since almost all one-pregnancy Rh-incompatible families and a large proportion of two-pregnancy families of this type are not yet sensitized (Glass, 1949), the reason for a lower mean age in the Rh NS series is apparent.

METHOD

Plan of Tabulation

The basic data have been tabulated in Appendix Tables I-VI. It will be necessary to refer to these to comprehend the statistical analyses, and the analytical tables. For the purpose of ready reference, the latter are indicated by section letters and numerals.

Plan of Analysis

The plan of analysis included:

(1) Comparison of the *observed* ABO distribution of mothers, fathers, and offspring, and their combinations, with the *expected* values for each series. Expected values are based on the assumption of a Hardy-Weinberg equilibrium, taking into account the racial composition of the sample and the ABO allele frequencies for U. S. Whites and Negroes, from Glass and Li (1953).

The expected numbers for each cell were computed separately for Whites and Negroes and then summed to yield cell expectancies. For example, the expected frequency of O × A matings Rh NS series =

$$\sum \{[r^2(p^2 + 2\,pr) \times \text{total Negro matings}] + [r^2(p^2 + 2\,pr) \times \text{total White matings}]\}$$

When the following allele frequencies are substituted for p, q, and r:

	Negroes	Whites
$p = I^A$	.180	.248
$q = I^B$	.132	.078
$r = I^O$	.688	.674

and 156, for total Negro matings, and 2157, for total white matings, the equation equals 408.3 matings or .17652 of all matings, after adjustment of the total is made for rounding the final integer. Likewise, the offspring of O × A matings are apportioned as:

$$\text{A offspring of O} \times \text{A, Rh NS} = [pr^2(p + r) \times \text{total Negro offspring} + pr^2(p + r) \times \text{total white offspring}]$$

$$\text{O offspring O} \times \text{A, Rh NS} = [pr^3 \times \text{total Negro offspring} + pr^3 \times \text{total white offspring}]$$

Computations were made similarly for the expected numbers of other matings, offspring, and parent-offspring combinations (appendix Tables V, VI and all other Hardy-Weinberg expectancies).

Complete tables with formulae, total frequency distributions, and computation for each cell are available and deposited in Welch Library.

It should be noted that the figures for U. S. whites are actually an average of frequencies for New York City (Tiber) and North Carolina (Snyder) and are in all likelihood representative of the Baltimore area. Moreover, they are based on 30,000 individuals and have almost no sampling error. On the other hand, while the frequencies for Negroes, derived from a Baltimore paternity suit series (cases of mother and offspring typed in connection with paternity suits at law), are representative of the Baltimore area and are similar to figures given for North Carolina and New York Negroes (Glass and Li, 1953), they are based on a relatively small sample (600 individuals) and involve some sampling error. However, since the Negro group represents such a small fraction of the population expectancy, attachment of a sampling error to this group would not alter the expected values to any degree.

(2) Comparison of population segments within each series.

(3) Comparison of the *observed* ABO distribution of the Rh S series with the *observed* ABO distribution of the Rh NS series for each population segment and set of combinations.

Note that comparisons of types (1) and (2) may be classified as comparisons *within each series*. Type (3) constitutes comparisons *between the two series* (Rh NS and Rh S).

TABLE A1. COMPARISON OF OBSERVED AND EXPECTED ABO DISTRIBUTIONS IN SUBGROUPS OF THE Rh NS SERIES AND THE Rh S SERIES*

	d.f.	Rh NS		Rh S	
		χ^2	P	χ^2	P
Mothers (Table II)	3	4.704	>.10	24.305	<.001
Fathers (Table II)	3	8.145	<.05	21.779	<.001
Offspring Total (Table III)	3	3.530	>.30	50.637	<.001
Male	3	2.204	>.50	26.482	<.001
Female	3	1.366	>.70	25.631	<.001
Mother-Offspring Combinations, m.a.	13	32.781	<.01	172.615	<.001
Father-Offspring Combinations, m.a.	13	34.408	<.01	74.657	<.001
Matings	15	32.117	<.01	88.991	<.001
Mating-Offspring Combinations	39	71.956	<.001	288.788	<.0001

* Comparisons of the observed and expected ABO distributions were also made using:
Mothers derived from M-O combinations for each series
Fathers " " F-O " " " "
Mother-Offspring combinations based on s.a. for each series
Father-Offspring " " " " " " "
These yielded a similar pattern of differences and levels of statistical significance to those tabulated above.

Reliability of Sampling

Internal consistency of the samples has been tested by Fisher's method, according to the procedure described by Dobson and Ikin (1946). All χ^2 values obtained were non-significant. Consequently, it may be assumed that the sampling is satisfactory.

Genetic formulae, statistical methods, and significance levels were computed in accordance with the accepted recommendations for standard procedures (Li, 1955; Cochran, 1952; Snedecor, 1950; Fisher, 1953).

RESULTS

For simplicity the results of the analysis of the data of Appendix Tables I–VI are presented below in tabular and outline form with brief, summarizing comments.

The following abbreviations will be used:

Symbol	Meaning
+	Excess of
−	Deficiency of
i	ABO-incompatible
c	ABO-compatible
NSD	No significant difference or not significantly different (i.e., not statistically significant)
SS	Statistically significant
M-O	Mother-offspring
F-O	Father-offspring (likewise, A-O indicates A mother-O offspring or A father-O offspring)
-x-	Mating Category listed as Female ABO blood group × Male ABO blood group
s.a.	Single ascertainment
m.a.	Multiple ascertainment
CPR	Cross-product ratio

Analysis of the Complete ABO Distribution of Population Segments and their Combinations

A. Comparisons within each Rh series

1. Comparisons of population subgroups with expectancy

To determine whether the ABO distributions of the Rh NS and Rh S series fit the Hardy-Weinberg expectancies, each population segment and set of combinations is compared with the theoretical expected values, for each series separately (Table A1).

2. Comparison between mothers and fathers *within each Rh series*

If, as is commonly assumed (Waterhouse and Hogben, 1947; Johnstone, 1955; Matsunaga and coworkers, 1955, 1956, 1958, 1959; Reed, 1956), there is a similar distribution of adult males and adult females among the four ABO blood groups in the general population, then it would be expected that, in the absence of selective forces, mothers and fathers within the same series (Rh NS or Rh S) would have a similar blood group distribution and that male and female

TABLE A2A. COMPARISON OF THE ABO BLOOD GROUP DISTRIBUTION OF MOTHERS WITH FATHERS WITHIN EACH SERIES

	Rh NS Series		Rh S Series	
	Mothers vs. Fathers		Mothers vs. Fathers	
	%	%	%	%
From M-O, F-O Combinations (Table V)*				
O	44.97	43.08	41.72	49.05
A	38.59	40.24	45.24	39.64
B	12.51	12.48	8.99	8.64
AB	3.92	4.20	4.04	2.68
Total Distribution:	$\chi^2_3 = 2.334$, .70 > P > .50		$\chi^2_3 = 15.473$, .01 > P > .001	

* Comparative distributions for Mothers and Fathers derived *from Matings*, which can be obtained directly from Table II, reveal a similar pattern to those from combinations tabulated above.

Rh NS Series: $\chi^2_3 = 1.598$, .70 > P > .50

Rh S Sereies: $\chi^2_3 = 18.914$, P < .001

TABLE A2B. COMPARISON OF PROPORTION OF TOTAL* OFFSPRING PRODUCED BY MOTHERS OF THE DIFFERENT ABO GROUPS WITH THAT OF FATHERS OF CORRESPONDING BLOOD GROUPS

Parental Blood Group	Rh NS Series				Rh S Series			
	From Matings (Table II)		From M-O and F-O Combinations (Table V)		From Matings (Table II)		From M-O and F-O Combinations (Table V)	
	Mothers %	Fathers %	Mothers %	Fathers %	Mothers %	Fathers %	Mothers %	Fathers %
O	44.27	42.12	44.81	42.48	41.25	48.46	42.39	49.07
A	39.06	40.54	38.80	40.89	47.15	39.71	43.63	39.43
B	12.99	12.89	12.36	12.88	9.54	9.57	9.81	9.15
AB	3.67	4.44	4.03	3.75	4.21	2.26	4.17	2.34
	$\chi^2_3 = 7.832$, .05 > P > .02		$\chi^2_3 = 4.070$, .30 > P > .20		$\chi^2_3 = 62.242$, P < .001		$\chi^2_3 = 34.675$, P < .001	

* Offspring are classified on the basis of *parental* blood groups, *not* their own blood groups.

parents of the same ABO blood group would produce a similar proportion of the total offspring (Tables A2a and A2b).

B. Comparisons *between series* (Rh NS and Rh S) of the ABO distributions of corresponding population segments and their combinations.

To determine whether the ABO patterns of the Rh NS and Rh S series are similar, the ABO distributions of corresponding population segments (mothers, fathers, etc.) and of sets of combinations (mother-offspring, matings, etc.) are compared between the two series (Table B).

TABLE B. COMPARISON BETWEEN SERIES: Rh NS VERSUS Rh S SERIES

Population Segment	d.f.	χ^2	P	Deviation[1] in ABO Distributions of Rh S Series when Compared to Rh NS Series
				Deficiency of:
Mothers (Table II)	3	13.210	$<.01$	*i* O and B mothers
Fathers (Table II)	3	26.160	$<.001$	*i* B and AB fathers
Offspring (Table III)				
Total	3	38.074	$<.001$	*i* B and AB offspring
Male	3	18.686	$<.001$	*i* B and AB offspring
Female	3	20.135	$<.001$	*i* B and AB offspring
Combinations				
M-O Combinations				
s.a. (Table V)	13	119.063	$<.001$	all *i* combinations
m.a. (Table V)	13	165.042	$<.001$	all *i* combinations
F-O Combinations				
s.a. (Table V)	13	43.483	$<.001$	all *i* combinations except AB-A and AB-AB
m.a. (Table V)	13	71.752	$<.001$	all *i* combinations except AB-AB contributing 1.3 to total χ^2.
Matings (Table VI)	15	92.268	$<.001$	all *i* categories
Mating-Offspring (Table VI)	39	207.327	$<.001$	Deficiency most marked in *i* combinations O × A-A; O × B-B; and O × AB-B

[1] For simplicity of comparison with analysis in Section A pattern of deviation is given in terms of ABO-incompatible (*i*) components.

[2] Statistical tests were also performed on mothers, fathers, and offspring (total, and male and female separately) derived from M-O combinations (s.a. and m.a., Table V) and F-O combinations (s.a. and m.a., Table V). All revealed a similar pattern of deviations attaining less than the .001 level of significance except the following groups of *female offspring:*

from M-O (s.a.) and (m.a.) $.01 > p > .001$
from F-O (s.a.) $.05 > p > .02$

TABLE C1A. COMPARISON OF OBSERVED AND EXPECTED ABO-INCOMPATIBLE COMBINATIONS IN THE Rh NS SERIES

Combinations	Total No.	ABO-Incompatible Combinations				χ^2_1	P
		Observed		Expected			
		No.	Per Cent	No.	Per Cent		
Mother-Offspring (Table V)							
m.a.*	3577	820	22.92	746	20.85	9.374	$<.01$
s.a.*	3037	697	22.95	633	20.85	8.379	$<.01$
Father-Offspring (Table V)							
m.a.	2828	1064	37.62	1046	36.99	.492	$>.30$
s.a.	2284	848	37.13	845	36.99	.020	$>.80$
Matings (Table VI)	2313	898	38.82	827	35.76	9.317	$<.01$
Mating-Offspring (Table VI)	2887	636	22.03	598	20.73	2.981	$>.05$

m.a. = multiple ascertainment per family; s.a. = single ascertainment per family.

TABLE C1B. COMPARISON OF OBSERVED AND EXPECTED ABO-INCOMPATIBLE COMBINATIONS IN THE Rh S SERIES

Combinations	Total No.	ABO-Incompatible Combinations				χ_1^2	
		Observed		Expected			
		No.	Per Cent	No.	Per Cent		
Mother-Offspring (Table V)							
m.a.*	2998	355	11.84	623	20.80	145.919	<.001
s.a.*	1335	133	9.96	278	20.80	94.879	<.001
Father-Offspring (Table V)							
m.a.	2645	839	31.72	978	36.96	31.154	<.001
s.a.	1158	352	30.40	428	36.96	21.450	<.001
Matings (Table VI)	1383	359	25.96	495	35.78	57.890	<.001
Mating-Offspring (Table VI)	2778	316	11.38	576	20.75	148.440	<.001

* m.a. = multiple ascertainment per family; s.a. = single ascertainment per family.

TABLE C2. COMPARISON RATIOS OF Rh NS AND Rh S SERIES AS TO PROPORTIONS OF ABO-INCOMPATIBLE AND ABO-COMPATIBLE COMBINATIONS AND OFFSPRING GROUPINGS

Set of Segments in Comparison (RhNS:RhS)	Cross Product Ratio[1] (X)	95 Per Cent Confidence Limits	Departure of CPR from unity	
			χ_1^2	P
Mother-Offspring (Table V)	2.214	1.933–2.535	132.212	<.001
Father-Offspring (Table V)	1.298	1.162–1.451	21.118	<.001
Matings (Table VI)	1.810	1.564–2.096	63.111	<.001
Offspring Groupings (Table VI)				
i offspring; *c* offspring	2.201	1.901–2.549	111.454	<.001
Offspring of *i* categories; offspring of *c* categories	1.8490	1.651–2.070	113.652	<.001

[1] Cross Product Ratio =

$$\frac{(i \text{ combinations or } i \text{ grouping RhNS})(c \text{ combinations or } c \text{ grouping RhS})}{(i \text{ combinations or } i \text{ grouping RhS})(c \text{ combinations or } c \text{ grouping RhNS})}$$

C. ABO-incompatible versus ABO-compatible combinations *within Rh series* and *between Rh series*

In the total distributions represented in sections "A" and "B" above, the ABO blood groups and blood group combinations appear to depart from expectation depending upon maternal-fetal ABO-compatibility (Chart 2).

To clarify these findings the various blood group combinations are pooled into two classifications, the ABO-incompatible and ABO-compatible types (Chart 2) and examined as follows:

Table C1. Comparison of the observed number of ABO-incompatible types with the Hardy-Weinberg (H-W) expectancies *within each Rh series.*

Table C2. Comparisons *between Rh series* by estimation of the cross product ratios of the proportion of ABO-incompatibles in the Rh NS series and the pro-

portion in the Rh S series for corresponding combinations (Woolf, 1955). The statistical significance of the cross product ratios depends on departure from unity.

Tables C3. Comparisons with H-W expectancy *within* Rh series and *between* Rh series, as to the distribution of ABO-incompatible and ABO-compatible offspring of each parental type (Table C3a) and each mating category (Table C3b) capable of producing both ABO-incompatible and ABO-compatible offspring.

Only those phenotypic parent groups and mating categories capable of producing both ABO-incompatible and ABO-compatible offspring are included. However, while O × AB matings, which can produce only ABO-incompatible offspring, were excluded, no attempt was made to distinguish between homozygosity or heterozygosity in A or B fathers in other tabulated categories. Therefore, some of the O × A matings might well be of genotype OO × AA and thus incapable of producing ABO-compatible offspring.

The *i*:*c* ratios are given in terms of numbers of individuals (observed and expected) rather than in the form of ratios based on unity. This affords a direct comparison between observation and expectancy.

D. Reciprocal matings

A comparison of the reciprocal mating categories involving one parent of blood group "O" necessarily constitutes a comparison of ABO-incompatible (or potentially incompatible) mating categories with ABO-compatible mating categories containing parents of the same ABO phenotypes. The strategic mating categories (mother × father) are O × A and A × O; O × B and B × O; O × AB and AB × O.

It will be assumed [as was done in the comparison of the blood group distribution of mothers versus fathers (A,2)] that there are equal proportions of male and female adults of corresponding blood groups and therefore that an equal number of each category of any pair of reciprocal mating categories is to be expected. The possible role of a differential sex ratio (Sanghvi, 1951; Johnstone, 1954; Cohen and Glass, 1956, 1959b) among the various blood groups and of differential selection in infancy and childhood (Struthers, 1951) cannot be ruled out. However, for the present, it is better to choose the simplifying assumption.

The following comparisons are made:

Table D1. Relative frequency of families of reciprocal mating categories, in terms of the ratio "ABO incompatible category: ABO compatible category."

Tables D2a,b. Comparisons within and between series involving blood group distribution of offspring (O offspring and offspring not O) of the categories in "D1" above.

SUMMARY OF RESULTS

A consistent pattern emerges from the analyses of the complete ABO distribution of mothers, fathers, and offspring (population segments) and their combinations within series (A) and between series (B), the pooled ABO-incompatible and pooled ABO-compatible combinations (C), and the reciprocal matings (D)

TABLE C3A. DISTRIBUTION OF OFFSPRING OF POTENTIALLY ABO-INCOMPATIBLE PARENTAL TYPES (TABLE VI)

Parental Types		Comparisons With Expectancy Within Each Series		Comparisons Between Series			
		Rh NS Series *i* offspring:*c* offspring	Rh S Series *i* offspring:*c* offspring	Cross Product Ratio[1]	95 Per Cent Confidence Limits	Departure of CPR from Unity	
						χ_1^2	P
Mothers							
O	Obs.	436:826 NSD	228:932 P < .001	2.158	1.637–2.844	29.795	< .001
	Exp.	410:852	377:783				
A	Obs.	100:1051 NSD	46:1169 P < .001	2.418	1.779–3.287	31.761	< .001
	Exp.	93:1058	98:1117				
B	Obs.	100:258 NSD	42:240 P < .001	2.215	1.483–3.307	15.108	< .001
	Exp.	85:273	67:215				
Fathers							
A	Obs.	381:805 NSD	221:894 P < .001	1.915	1.582–2.318	44.368	< .001
	Exp.	390:796	367:748				
B	Obs.	171:198 NSD	70:187 P < .001	2.307	1.638–3.249	22.893	< .001
	Exp.	163:206	113:144				
AB	Obs.	84:30 NSD	25:34 P < .001	3.808	1.961–>5.0	15.594	< .001
	Exp.	81:33	42:17				

[1] Cross Product Ratio = $\frac{(i \text{ offspring Rh NS})(c \text{ offspring Rh S})}{(i \text{ offspring RhS})(c \text{ offspring Rh NS})}$

TABLE C3B. DISTRIBUTION OF OFFSPRING OF ABO-INCOMPATIBLE MATING CATEGORIES (TABLE VI)

Mating Category		Comparison with Expectancy Within Each Series		Comparisons Between Series			
		Rh NS Series *i* offspring:*c* offspring	Rh S Series *i* offspring:*c* offspring	Cross Product Ratio[1]	95 Per Cent Confidence Limits	Departure of CPR from Unity	
						χ_1^2	P
O × A	Obs.	283:231 NSD	182:230 P < .001	1.548	1.193–2.010	10.792	< .001
	Exp.	296:218	238:174				
O × B	Obs.	100:77 NSD	37:68 P < .001	2.387	1.450–3.930	11.694	< .001
	Exp.	94:83	56:49				
A × B	Obs.	71:76 NSD	33:40 .20 > P > .10	1.132	0.645–1.989	1.873	.20 > P > .10
	Exp.	78:69	39:34				
B × A	Obs.	98:88 NSD	39:39 .20 > P > .10	1.114	0.656–1.890	1.589	.30 > P > .20
	Exp.	107:79	45:33				
A × AB	Obs.	28:20 NSD	13:21 .20 > P > .10	2.262	0.921–>5.0	3.167	.10 > P > .05
	Exp.	24:24	17:17				
B × AB	Obs.	2:6 NSD	3:6 .50 > P > .30	.667	0.080–>5.0	0.141	.80 > P > .70
	Exp.	4:4	4.5:4.5				

[1] Cross Product Ratio = $\frac{(i \text{ offspring, RhNS})(c \text{ offspring, Rh S})}{(i \text{ offspring, RhS})(c \text{ offspring, Rh NS})}$

Expected values are rounded to nearest whole number.

TABLE D1. NUMBER OF FAMILIES OF RECIPROCAL ABO-INCOMPATIBLE AND ABO-COMPATIBLE CATEGORIES (TABLE VI)

Matings	Comparisons within Each Series				Comparisons Between Series			
	Rh NS		Rh S		Cross Product Ratio†	95 Per Cent Confidence Limits	x_1^2	P
	No. of Families	Observed Ratio*	No. of Families	Observed Ratio*				
O × A:A × O	405:405	1.00:1.00 (NSD)	197:274	0.72:1.00 (P < .001)	1.391	1.098–1.736	7.623	.01 > P > .001
O × B:B × O	135:118	1.14:1.00 (P > .30)	54:64	0.84:1.00 (P > .20)	1.356	0.875–2.102	1.853	.20 > P > .10
O × AB:AB × O	48:37	1.30:1.00 (P > .20)	6:22	0.27:1.00 (P < .01)	4.757	1.751–>5	9.355	.01 > P > .001

* Expected ratio of ABO-incompatible:ABO-compatible categories would be 1.00:1.00. "P" values in these columns refer to the significance of the difference between observed and expected ratios.

† Cross Product Ratio = $\frac{(i \text{ category Rh NS})(c \text{ category Rh S})}{(i \text{ category Rh S})(c \text{ category Rh NS})}$

TABLE D2A. COMPARISON OF BLOOD GROUP DISTRIBUTION OF OFFSPRING OF RECIPROCAL MATING CATEGORIES WITHIN EACH SERIES (TABLE VI)

Reciprocal Categories in Comparison Ratio	Total No. Offspring per Mating	Cross Product Ratio*	95 Per Cent Confidence Limits	χ_1^2	p
Rh NS Series					
O × A	514				
and		1.036	0.811–1.325	0.081	.80 > p > .70
A × O	515				
O × B	177				
and		1.261	0.805–1.975	1.027	.50 > p > .30
B × O	136				
Rh S Series					
O × A	412				
and		−0.489	0.474–0.795	13.701	< .001
A × O	529				
O × B	105				
and		−0.671	0.301–0.868	6.169	.02 > p > .01
B × O	128				

* Cross Product Ratio = $\frac{\text{(not-O offspring } i \text{ mating category)(O offspring } c \text{ mating category)}}{\text{(not-O offspring } c \text{ mating category)(O offspring } i \text{ mating category)}}$

TABLE D2B. COMPARISON BETWEEN SERIES OF BLOOD GROUP DISTRIBUTION OF OFFSPRING BY MATING CATEGORY (TABLE VI)

Mating Category in Comparison Ratio (Rh NS and Rh S Series)	Cross Product Ratio*	95 Per Cent Confidence Limits	χ_1^2	p
i categories				
O × A	1.548	1.193–2.010	10.792	.01 > p > .001
O × B	2.387	1.450–3.930	11.694	< .001
c categories				
A × O	0.916	0.719–1.169	0.494	.50 > p > .30
B × O	0.967	0.597–1.568	0.018	.90 > p > .80

* Cross Product Ratio = $\frac{\text{(not-O offspring, Rh NS)(O offspring, Rh S)}}{\text{(not-O offspring, Rh S)(O offspring, Rh NS)}}$

for each mating category specified.

In both the *Rh-sensitized* (Rh S) series and the *Rh-nonsensitized* (Rh NS) series the ABO distribution of every population segment and every set of combinations (mother-offspring, father-offspring, matings and mating-offspring) is different from expectancy, significantly in the former series ($p < .001$), though not always in the latter series [Table A1].

The *Rh S series* shows a *deficiency of* those elements that are *ABO-incompatible* or most markedly associated with ABO-incompatibility, whereas the Rh NS

series shows a trend toward an *excess of ABO-incompatible* types and combinations [Chart 2, Table 1 (ABO-compatibility status); Appendix Tables II, III, V, VI (*observed distributions*)].

In view of the opposite direction of the deviations of the Rh NS and Rh S series from the Hardy-Weinberg expectancies, the contrast in the ABO distributions of the two series is sharpest when comparisons are made directly between them. Each of the comparisons involving population segments or sets of combinations shows a highly significant difference between the two series [Table B and Basic Tables II, III, V, VI].

That the aberrant ABO distributions are not a function of specific parental or offspring blood groups per se but in fact result from the ABO-compatibility status of combinations is illustrated in several ways.

First, the comparison of mothers with fathers within each series shows differences in their respective ABO-distributions (Table A2a) as well as differences in the proportion of total offspring produced by mothers and fathers of corresponding blood groups within each series (Table A2b). In the Rh S series there is a smaller proportion of O mothers (potentially ABO-incompatible, Table 1) and a larger proportion of A (relatively ABO-compatible) and AB (always ABO-compatible) mothers as compared to the frequencies of fathers of the same blood groups—and opposite ABO-compatibility status—$P < .01$. Likewise, in the Rh S series there is a smaller proportion of total offspring produced by O mothers and a larger proportion produced by A and AB mothers as compared to the proportion of total offspring produced by offspring of fathers of corresponding blood groups ($P < .001$).

The Rh NS series shows a tendency in the opposite direction both in the maternal-paternal ABO-distribution and in the relative proportions of offspring produced by the same maternal and paternal blood groups, although borderline significance is attained only in the offspring derived from the tabulation of matings.

Secondly, direct evidence of the role of ABO-compatibility status in the observed pattern comes from the comparison of the pooled observed ABO-incompatible combinations with the Hardy-Weinberg expectancies (Tables C1a and C1b). The *deficiency of ABO-incompatible* combinations in the *Rh S* series is significant at the .001 level in all comparisons. In the *Rh NS* series the *excess of ABO-incompatible* combinations attains statistical significance except for the father-offspring and mating-offspring combinations.

When comparisons are made directly between the *Rh NS* and *Rh S series* (Table C2), all sets of combinations (mother-offspring, father-offspring, matings, and offspring groupings from matings) emphasize the significantly larger proportion of ABO-incompatibles in the former series ($P < .001$). Cross-product ratios range from almost 1.3 in father-offspring to more than 2.2 for mother-offspring and mating-offspring sets.

Thirdly, the proportions of ABO-incompatible and ABO-compatible offspring produced by mothers of each blood group and fathers of each blood group capable of producing both types of offspring (Table C3a), as well as by each mating

category (Table C3b) capable of producing both ABO-incompatible and ABO-compatible offspring, confirm the overall pattern. In the Rh S series O, A, and B mothers and A, B, and AB fathers (Table C3a) and O × A and O × B matings (Table C3b) all show a *significantly smaller* proportion of ABO-incompatible offspring than expected. In the Rh NS series a tendency toward an excess of ABO-incompatible offspring (not statistically significant) is observed when classification is based on individual parental types, although the individual mating categories show some irregularities.

Accordingly, the contrasting pattern in the Rh NS and Rh S series is reflected in comparisons of the relative proportions of ABO-incompatibles in the two series. The cross-product ratios for offspring of corresponding parental types indicate approximately twofold or greater (1.915–3.808) differences between the two series. The trend shown by the offspring of mating categories is similar but not so striking, with significance in comparisons between series only in the O × A and O × B categories, which yield cross-product ratios of 1.5 to >2.3.

Fourthly, the *reciprocal mating* categories, both in their deviations from the expected 1:1 ratio of relative frequency and in the blood group distributions of their offspring, show a significant deficiency of ABO-incompatibles in the Rh S series [deficiency of O × A, and O × AB matings (Table D1) and deficiency of A offspring in O × A matings and B offspring in O × B matings (Table D2a) relative to the findings in their reciprocal mating categories]. In the Rh NS series there is a trend (not statistically significant) in the opposite direction. The contrast in the relative frequency of reciprocal mating categories of the two series (Table D1) is highly significant for the O × A:A × O group (cross-product ratio = 1.39) and for the O × AB:AB × O group (cross-product ratio = 4.76).

The striking ratio in the O × AB:AB × O might well be attributable to the fact that the O × AB category is the only ABO-incompatible mating category that is unconditionally ABO-incompatible—no ABO-compatible offspring can be produced. In the collection of families of Rh-sensitized women reported by Levine (1958) the ratio of O × AB:AB × O matings was 1:59 when transfused mothers were eliminated. While no attempt has been made in the Rh S series of the present study to separate all those matings where Rh sensitization may have been caused or influenced by factors other than Rh-incompatible pregnancies in the particular mating recorded, it is noteworthy that all of the six O × AB matings listed in the Rh S series involved women who had either been transfused (families 1279, 1303, and 1542), or had been married at least once prior to the current marriage (families 866, 922, and 1490).

In regard to the blood group distributions of offspring, the comparisons between reciprocal categories reach levels of statistical significance only in the Rh S series, although the opposing trends in the two series are apparent from the cross-product ratios of ABO-incompatibles to ABO-compatibles (more than unity in the Rh NS and less than unity in the Rh S—Table D2a). Comparisons between series for offspring distribution in each mating category reveal, in the ABO-incompatible categories O × A and O × B, a marked contrast between the two series, whereas in the reciprocal ABO-compatible categories (in which both

alternative types of offspring O and not-O are alike ABO-compatible) there are no significant differences between the two series in the blood group distribution of the offspring (Table D2b).

A more detailed analysis of the reciprocal mating categories has been made and is to be presented in the near future. It is noteworthy that when the data are analyzed grouping observations by total number of pregnancies per mating and by pregnancy order, the findings are consistent with the pattern indicated by the pooled groups reported here. This suggests that the ABO blood group differences between the two series cannot be attributed to pregnancy order, or maternal age.

DISCUSSION

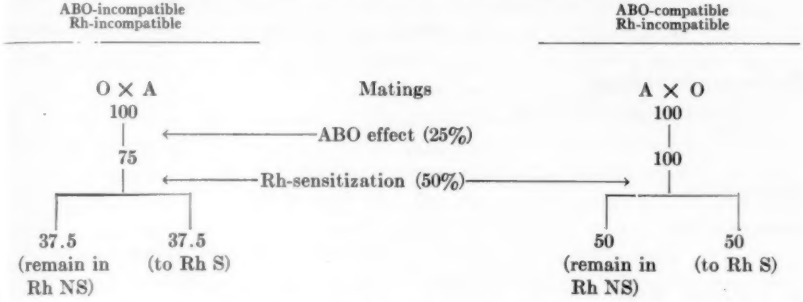
By approaching the data from many points of view the inner consistency of the ABO-Rh relationship is repeatedly demonstrated. Even the nonsignificant trends in the Rh NS series seem fairly well established by means of repeated appearance under different methods of analysis. Clearly, the ABO distributions of the fertile Rh-incompatibly mated population samples, as represented by the Rh NS (not sensitized) and Rh S (sensitized) series not only deviate significantly from one another, but, even more definitively, deviate in opposite directions from theoretical (Hardy-Weinberg) expectancies.

The highly significant deficiency of ABO-incompatibles in the Rh S series of this study is consistent with the findings of other investigators who have examined the families of Rh-sensitized women (Levine, 1945, 1958; Wiener, 1945; Heistö, 1955; Reepmaker, 1956 and others). The opposite tendency, toward an excess of ABO-incompatibles, here seen in the Rh NS series, is, on the other hand, a deviation opposite in direction not only from that in the Rh S series of this study, but also from those in every other Rh-sensitized, Rh-mixed (Waterhouse and Hogben, 1947, Johnstone, 1954a), or Rh-compatibly mated (Matsunaga, 1959) series thus far reported.

Several theories have previously been proposed to explain Levine's original observation of a deficiency of ABO-incompatibly mated Rh-negative mothers of erythroblastotic offspring. All such theories will explain a deficiency of ABO-incompatible types in the Rh-sensitized series. The use of a nonsensitized Rh-incompatibly mated series (Rh NS) now makes possible a decision as to the correct hypothesis.

The deviations from expectation observed in the present data are not explicable on the basis of simple negative selection against A- and B-incompatible types, as Fisher suggested (1944, according to Race, MRC Memorandum No. 27). Such a theory would account for the aberrant ABO distribution in Rh unselected and Rh-compatibly mated samples, but, because it postulates that ABO-incompatibility and Rh-incompatibility act independently of one another, this theory would also require that the Rh NS series show a deficiency of ABO-incompatibles similar to that in the Rh S series. For example, if ABO-incompatibility selected against 25 percent of the ABO-incompatible matings and Rh-incompatibility independently caused 50 percent of Rh-incompatible matings to

become Rh-sensitized, the following might be expected in the reciprocal ABO-incompatible O × A and ABO-compatible A × O matings:



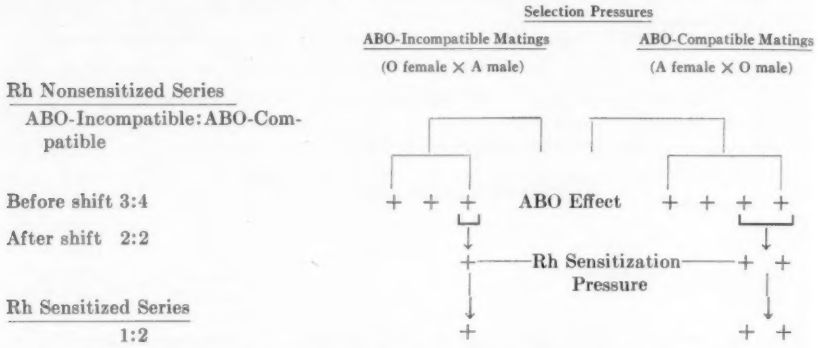
Thus the ratio of ABO-incompatible:ABO-compatible matings would be the same in the Rh NS and the Rh S series, 37.5:50, or .75:1.00. That is to say, both the Rh NS and Rh S series would show a relative deficiency—the same deficiency—of ABO-incompatible types.

Still another proposed explanation for the marked deficiency of ABO-incompatible matings among Rh-sensitized mothers attributes the phenomenon to inherent, most probably genetic, differences in the ability to produce antibodies, i.e., to differences among mothers in "sensitizability." According to this hypothesis, Rh-sensitized mothers would be not only good Rh antibody-makers but also good anti-A or anti-B makers also. Thus by selection for Rh-sensitized mothers, selection would have been made simultaneously for a strong ABO-incompatibility effect.

The Rh NS series, on the other hand, since it is a group selected for nonsensitization to Rh, would then be expected to be composed of poorly sensitizable or nonsensitizable mothers, showing neither ABO nor Rh response. However, since all mothers are classified as nonsensitized until they produce antibodies, the Rh NS series must be considered in a dual role: it is the "parent" population of the Rh S series as well as a comparable "sister" population. As the parent population, the Rh NS series must contain some families which are potentially destined for the Rh S series, and these families not being true "nonsensitizables," would be expected to show an ABO-incompatibility effect similar to that of the Rh S series. The resultant Rh NS series should therefore show "no difference" from expectation or possibly a slight trend toward a *deficiency* of ABO-incompatibles, as in the Rh S series. On the basis of the sensitizability hypothesis, therefore, one would not at all expect a consistent trend toward an *excess* of ABO-incompatibles in the Rh NS series, such as actually occurs.

The only current theory that can explain both the deficiency of ABO-incompatibles in the sensitized Rh-incompatibly mated population (Rh S series) and at the same time an excess of ABO-incompatibles in the nonsensitized Rh-incompatibly mated population is one that postulates an interaction of ABO-incompatibility and Rh-incompatibility, most probably involving a protective

CHART 3. The preferential shift in Rh-incompatible matings of ABO-compatible matings in comparison with ABO-incompatible matings (after Cohen and Glass, 1959a)



Each "+" represents an equal number of matings.

The primary ABO effect is represented by the reduction in number of +'s (fertile matings) representing the ABO-incompatible matings as compared with the ABO-compatible matings (i.e. 3:4).

The differential in Rh sensitization pressure is represented by the number of +'s shifted relative to the number belonging to the mating category before the shift (i.e., 1/3 for O × A matings; 2/4 for A × O matings).

action of ABO-incompatibility against the Rh effect. A differential Rh-sensitization pressure favoring ABO-incompatible over ABO-compatible matings would produce a preferential shift of ABO-compatible matings from the Rh NS series into the Rh S series. As a consequence, a deficiency of ABO-incompatible matings would occur in the Rh S series, whereas a backlog (or excess) of ABO-incompatible matings would be left in the Rh NS series [an hypothetical example of this shift appears in Chart 3 (Fig. 1, Cohen and Glass, 1959a)].

By manipulating the assumed values for ABO and Rh selection pressures, it may readily be demonstrated that *a difference in negative Rh selection pressure superimposed on the negative AB selection pressure can actually yield either a deficiency, excess, or equality* of the ABO-incompatibly mated families in comparison with their reciprocal ABO-compatibly mated families in the Rh NS series, while the Rh S series still shows a deficiency. Some of the factors that could determine which of the three relations—deficiency, excess, or equality—will occur are the following:

(1) The degree of negative selection against A and B imposed on ABO-incompatibles in comparison with ABO-compatibles—from the effect of ABO-incompatibility alone.

(2) The difference between the Rh sensitization pressures for ABO-incompatibles and ABO-compatibles.

(3) The magnitude or level of Rh sensitization pressures.

(4) The magnitude of Rh sensitization pressures relative to the primary negative ABO selection pressure in (1) above.

(5) The relative size of (a) the differential between the Rh-sensitization pressures and (b) the AB negative selection pressure.

The findings of this investigation suggest an interaction of these five components of the total selection pressure at such levels as to yield a marked *deficiency* of ABO-incompatibles in the Rh-sensitized series and a slight borderline *excess* of ABO-incompatibles in the Rh-nonsensitized series. This difference in degree of the deviations as well as direction of the deviations in the two series is apparent when the effects of the component selection pressures for each series are considered. In the Rh S series both the simple negative selection against A- and B-incompatibles in early pregnancy and the protective action of ABO-incompatibility against Rh sensitization contribute to the size of the deficiency of ABO-incompatibles. On the other hand, in the Rh NS series, these two components of selection oppose one another. Selection against ABO-incompatibles in early pregnancy tends to diminish the excess of ABO-incompatibles produced by the protective action of ABO-incompatibility against Rh-sensitization.

From the present observations of the ABO-Rh interaction in the Rh NS and Rh S series, the probable effects upon fetal survival and differential blood group distribution of offspring in the total Rh-incompatibly mated segment of the population may be postulated. The concept has been illustrated in a theoretical example [see Chart 4 (Figure 2, Cohen and Glass, 1959a)] in which there is no separation into nonsensitized and sensitized series, but instead the ultimate fate of all Rh-incompatible zygotes is considered.

The illustration is based on the following simplifying assumptions:

(1) ABO-incompatibility tends to manifest its deleterious effects primarily in early pregnancy, sometimes so early that pregnancy is unrecognized (Brambell et al., 1951; Waterhouse and Hogben, 1947; Matsunaga 1955, 1956, 1958, 1959). AB hemolytic disease is relatively so rare (1 in 1100 pregnancies) that it need not be considered in comparison with the early ABO effect (1 in 25 pregnancies).

(2) Rh incompatibility manifests its harmful effects in late pregnancy and early neonatal life, and not at all in the first half of pregnancy (Overstreet, Trent, Hunt, and Lucia, 1947; Glass, 1949).

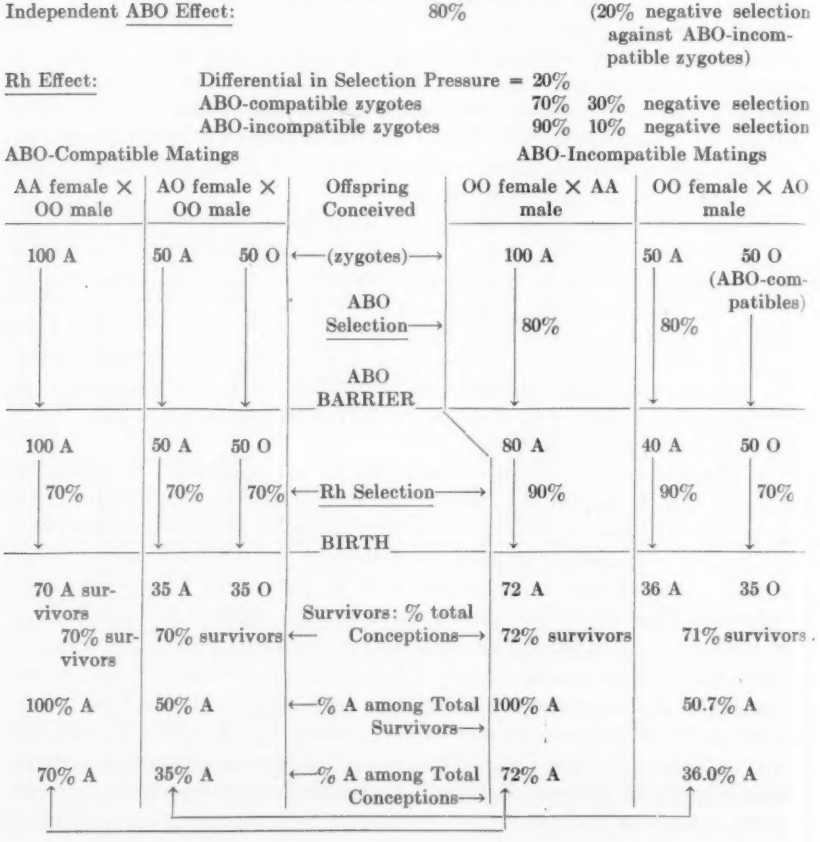
The effect of simple ABO-incompatibility in early pregnancy is postulated to be such that 20 percent of ABO-incompatible zygotes are eliminated. Superimposed on this effect is a 20 per cent differential in Rh selection pressure favoring the ABO incompatible zygotes; 70 percent of the ABO-compatible Rh-incompatible zygotes survive as compared to 90 percent of ABO-incompatible Rh-incompatibles, which have passed the early 20% negative A-B selection.

As a consequence of these selection pressures the model yields more survivors in ABO-incompatible matings than in ABO-compatible matings: 72 percent survivors in OO × AA matings in comparison with 70 percent in AA × OO, 71 percent in OO × AO as compared with 70 percent in AO × OO. In addition, there is a higher percentage of A survivors among total conceptions and among all survivors in the progeny of ABO-incompatible matings than in the progeny of ABO-compatible matings.

	Percentage of Type A	
	Among Total Conceptions	Among Survivors Only
OO × AA	72%	100% A } only type possible
AA × OO	70%	100% A } only type possible
OO × AO	36%	50.7%
AO × OO	35%	50.0%

Thus with reasonable combinations of ABO-selection pressures and differentials in Rh selection pressures for ABO-incompatible and ABO-compatible offspring it is apparent that the doubly incompatible zygotes, though selected

CHART 4. Opposing selection pressures in Rh-incompatible matings
(after Cohen and Glass, 1959a)
A simplified hypothetical example



against by two negative selection pressures (ABO and Rh), can actually show a higher rate of survivorship and a higher proportion of non-O survivors than the singly incompatible (ABO-compatible, Rh-incompatible) zygotes.

The statistical findings presented in this investigation and other studies (Grubb and Sjöstedt, 1955) support the view that the double heterozygote (ABO-incompatible, Rh-incompatible) is, in fact, superior in fitness to the single heterozygote (ABO-compatible, Rh-incompatible), as assumed in the model. The evidence is furthermore consistent with the view that the selective disadvantage of ABO-incompatibility in early pregnancy is counteracted by the selective advantage of ABO-incompatibility in protecting against the deleteriousness of Rh-incompatibility in later pregnancy (Cohen and Glass 1959a).

Several hypotheses have been advanced to account for the biological mechanism whereby ABO-incompatibility between mother and fetus can protect an Rh-negative mother against isoimmunization to Rh. For the most part the weight of evidence seems equally favorable to each of two biological mechanisms, competition between antigens (Wiener, 1945) and destruction of ABO-incompatible fetal erythrocytes, when they get into the maternal circulation, by the maternal anti-A or anti-B present there (Race, 1952; Levine, 1958, and others). Neither acquired tolerance (Owen et al., 1954), nor injections of ABO-incompatible Rh-incompatible and ABO-compatible Rh-incompatible blood (Stern, Davidsohn, and Masaitis, 1956), nor the clinical cases of combined AB and Rh hemolytic disease (Gunson, 1957) permit a definite choice between the two explanations. Moreover, since the two theories are not mutually exclusive, it remains possible, as a third alternative, that both are valid. It is feasible that the protective action of ABO-incompatibility may itself be mediated in more than one way and that quantitative as well as qualitative effects need to be considered.

That the ramifications of selective interaction involved in this phenomenon are numerous and are not to be regarded as completely resolved is apparent. In the present investigation of the ABO-Rh interaction, additional findings from the detailed analysis of reciprocal matings, including data on pregnancy wastage and the blood group distribution of offspring classified by pregnancy order and total number of pregnancies, remain to be presented. Emprical estimates and mathematical models of the protective action of ABO-incompatibility against the Rh effect are being formulated. We may hope that other populations will be studied by biometricians, epidemiologists, serologists, and clinicians, as well as by geneticists, for the purpose of clarifying the biological mechanisms involved, refining the estimates for the clinical outcome of pregnancies, and indicating the role of the dual incompatibility phenomena on evolution. The effect of Rh-incompatibility on Rh-rh frequencies in future generations has already been studied by Haldane (1942), Wiener (1942), Glass (1950), and Levine (1958). Nevertheless, it is even now apparent—though still too soon to make precise estimates—that the interaction ABO-incompatibility and Rh-incompatibility must be considered in evaluating the evolutionary trends of allele frequencies at both the ABO and the Rh loci.

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SUMMARY

1. The ABO distributions of Rh-nonsensitized and Rh-sensitized Rh-incompatibly mated population samples not only deviate significantly from one another, but also deviate in opposite directions from Hardy-Weinberg expectancies.

2. Several theories previously proposed to explain the deficiency of ABO-incompatibles in Rh-sensitized samples may be excluded through the utilization of an Rh-nonsensitized as well as an Rh-sensitized series. The only hypothesis which can explain simultaneously both the *deficiency* of ABO-incompatibles in the Rh-sensitized series and the *excess* of ABO-incompatibles in the Rh-nonsensitized series is an interaction between ABO-incompatibility and Rh-incompatibility, such that ABO-incompatibility tends to inhibit Rh-isoimmunization, and possibly also modifies its consequences.

3. The theoretical basis of the superiority of the double heterozygote (ABO-incompatible, Rh-incompatible) over the single heterozygote (ABO-compatible, Rh-incompatible) is considered along with hypothetical models to illustrate (a) the preferential shift of ABO-compatible matings from the Rh-nonsensitized series to the Rh-sensitized series; and (b) differential fetal survival and blood group distribution of offspring as a result of the ABO-Rh interaction.

4. The possible biological mechanisms underlying the ABO-Rh interaction and its possible impact on allele frequencies in future generations are discussed.

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TABLE 1. ABO BLOOD GROUP DISTRIBUTION OF WHITE AND NEGRO MOTHERS[1] OF BALTIMORE

	O				A				B				AB				Total Number		
	White		Negro		White		Negro		White		Negro		White		Negro				
	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	No.	Per Cent	White	Negro	Series
Rh NS Series	1109	43.5 ± 1.0	185	47.6 ± 2.5	1048	41.1 ± 1.0	108	27.8 ± 2.3	296	11.7 ± 0.6	83	21.3 ± 2.1	99	3.9 ± 0.4	13	3.3 ± 0.9	2552	389	2941
Rh S Series	564	40.5 ± 1.3	68	47.6 ± 4.2	651	46.8 ± 1.3	48	33.6 ± 4.0	125	9.0 ± 0.8	18	12.6 ± 2.8	52	3.7 ± 0.5	9	6.3 ± 2.0	1392	143	1535
Glass and Li		45.2		47.6		39.5		28.3		11.2		20.0		4.2		4.1			

Significance Tests: Comparison of observed ABO distribution with expectancy based on distribution of Glass and Li:

	White	*Negro*
Rh NS Series	$\chi^2_3 = 4.235$, $.30 > p > .20$	$\chi^2_3 = 0.948$, $.90 > p > .80$
Rh S Series	$\chi^2_3 = 32.279$, $p < .001$	$\chi^2_3 = 6.947$, $.10 > p > .05$

[1] All mothers tabulated in mother-offspring combinations and/or mating categories for whom race and blood group are known.

TABLE II. ABO BLOOD GROUP DISTRIBUTION OF PARENTS INCLUDED IN MATINGS OF Rh NS AND Rh S SERIES

	Series Total	O			A			B			AB			Total Offspring
		No. Parents	% Total Parents	No. of Offspring	No. Parents	% Total Parents	No. of Offspring	No. Parents	% Total Parents	No. of Offspring	No. Parents	% Total Parents	No. of Offspring	
Rh NS Series														
Mothers	2313	1008	43.58 ± 1.03	2242	923	39.90 ± 1.02	1978	294	12.71 ± 0.69	658	88	3.80 ± 0.40	186	5064
Fathers	2313	980	42.37 ± 1.03	2133	940	40.64 ± 1.02	2053	291	12.58 ± 0.69	653	102	4.40 ± 0.43	225	5064
Total Parents	4626	1988	42.97 ± 0.73		1863	40.27 ± 0.72		585	12.64 ± 0.49		190	4.11 ± 0.29		
Expectancy			45.3			38.7			11.8			4.2		
Rh S Series														
Mothers	1383	574	41.50 ± 1.32	1716	620	44.83 ± 1.34	1872	133	9.62 ± 0.79	397	56	4.04 ± 0.53	175	4160
Fathers	1383	677	48.95 ± 1.34	2016	544	39.33 ± 1.31	1652	128	9.26 ± 0.78	398	34	2.46 ± 0.42	94	4160
Total Parents	2766	1251	45.23 ± 0.95		1164	42.08 ± 0.94		261	9.44 ± 0.56		90	3.25 ± 0.34		
Expectancy			45.3			38.6			11.9			4.2		

TABLE III. ABO BLOOD GROUP DISTRIBUTION OF OFFSPRING OF MATINGS OF Rh NS AND Rh S SERIES

TABLE III. ABO BLOOD GROUP DISTRIBUTION OF OFFSPRING OF MATINGS OF Rh NS AND Rh S SERIES

Offspring	Grand Total	Total Known Blood Group	O Offspring		A Offspring		B Offspring		AB Offspring		Unknown Blood Group	
			No.	% Known Blood Group	No.	% Known Blood Group	No.	% Known Blood Group	No.	% Known Blood Group	No.	% Total Blood Group
Rh NS Series												
Females	2385	1384	621	44.87 ± 1.34	528	38.15 ± 1.31	177	12.79 ± 0.90	58	4.19 ± 0.54	1001	41.97 ± 1.01
Males	2457	1466	663	45.23 ± 1.30	555	37.86 ± 1.27	190	12.96 ± 0.88	58	3.96 ± 0.51	991	40.33 ± 0.99
Male + Female	4842	2850	1284	45.05 ± 0.93	1083	38.00 ± 0.91	367	12.88 ± 0.63	116	4.07 ± 0.37	1992	41.14 ± 0.71
Sex Unknown	222	37	12	32.43 ± 7.70	14	37.84 ± 7.98	6	16.22 ± 6.06	5	13.51 ± 5.62	185	83.33 ± 2.50
Total Offspring	5064	2887	1296	44.89 ± 0.93	1097	38.00 ± 0.90	373	12.92 ± 0.63	121	4.19 ± 0.37	2177	42.99 ± 0.70
Expectancy				45.3		38.7		11.8		4.2		
Rh S Series												
Females	1740	1258	637	50.64 ± 1.41	476	37.84 ± 1.37	115	9.14 ± 0.81	30	2.38 ± 0.43	482	27.70 ± 1.07
Males	1938	1373	694	50.55 ± 1.35	521	37.95 ± 1.72	121	8.81 ± 0.59	37	2.69 ± 0.44	565	29.15 ± 1.03
Male + Female	3678	2631	1331	50.59 ± 0.97	997	37.89 ± 0.95	236	8.97 ± 0.56	67	2.55 ± 0.31	1047	28.47 ± 0.74
Sex Unknown	482	147	73	49.66 ± 4.12	51	34.69 ± 3.93	16	10.88 ± 2.57	7	4.76 ± 1.76	335	69.50 ± 2.10
Total Offspring	4160	2778	1404	50.54 ± 0.95	1048	37.72 ± 0.92	252	9.07 ± 0.54	74	2.66 ± 0.31	1382	33.22 ± 0.73
Expectancy				45.3		38.6		11.9		4.2		

Expectancy based on U. S. population blood group frequencies (Glass and Li) and racial distribution of series.

TABLE IV. MEAN MATERNAL AGES BY ABO BLOOD GROUP OF MOTHERS

Series	O			A			B			AB			Total		
	No.	Mean Age	±S.E.	No.	Mean Age	±S.E.	No.	Mean Age	±S.E.	No.	Mean Age	±S.E.	No.	Mean Age	±S.E.
Rh NS	1294	27.045	±0.155	1156	27.393	±0.171	379	27.223	±0.305	112	27.888	±0.586	2941	27.237	±0.106
Rh S	612	28.423	±0.229	685	28.870	±0.224	136	28.118	±0.484	60	29.025	±0.743	1493	28.625	±0.149
T-tests: Rh NS vs. Rh S Series	t = 4.976 P < .01			t = 5.241 P < .01			t = 1.566 NSD			t = 1.201 NSD			t = 7.601 P < .01		

Significance Tests:	Rh NS Series	Rh S Series
Most deviant pair within series:	O vs. AB t = 1.390 NSD	B vs. AB t = 1.023 NSD
Other deviations within series:	O vs. A t = 1.505 NSD	O vs. A t = 1.395 NSD
		O vs. AB t = 0.778 NSD

TABLE VI. COMPARISON OF THE OBSERVED AND EXPECTED DISTRIBUTIONS OF MATINGS AND OFFSPRING

Category	Matings				Offspring													
	Rh NS		Rh S		O				A				B				AB	
					Rh NS		Rh S		Rh NS		Rh S		Rh NS		Rh S		Rh NS		Rh S	
	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.
ABO-compatible	1415		1024																	
O × O	420	479.9	317	287.1	518	599	634	577												
A × A	359	350.0	285	208.5	84	78.1	131	74.7	357	358.5	448	343.0								
B × B	25	33.0	28	20.2	5	9.0	18	8.9					23	34.3	49	33.6				
AB × AB	4	3.5	3	2.1					1	1.1	1	1.0	3	1.1	1	1.0	—	2.2	5	2.0
A × O	405	408.3	274	243.3	236	215.7	231	206.9	279	293.8	298	281.9								
B × O	118	123.9	64	74.7	67	72.6	62	70.5					69	81.6	66	79.2				
AB × O	37	41.4	22	24.8					24	25.9	26	25.0	24	25.9	30	25.0				
AB × A	33	35.1	25	21.0					22	21.9	17	21.0	14	9.3	12	8.9	10	12.6	17	12.1
AB × B	14	10.8	6	6.5					1	3.1	3	3.1	11	6.7	6	6.5	5	3.6	3	3.5
ABO-incompatible	898		359																	
O × A	405	408.3	197	243.3	231	215.7	230	206.9	283	293.8	182	281.9								
O × B	135	123.9	54	74.7	77	72.6	68	70.5					100	81.6	37	79.2				
O × AB	48	41.4	6	24.8					20	25.9	7	25.0	33	25.9	2	25.0				
A × B	117	103.9	40	62.2	35	25.9	13	25.0	41	35.2	27	33.9	27	29.0	16	28.0	44	39.3	17	38.0
B × A	143	103.9	37	62.2	43	25.9	17	25.0	48	35.2	18	33.9	45	29.0	22	28.0	50	39.3	21	38.0
A × AB	42	35.1	21	21.0					20	21.9	21	21.0	18	9.3	5	8.9	11	12.6	8	12.1
B × AB	8	10.8	4	6.5					1	3.1		3.1	6	6.7	6	6.5	1	3.6	3	3.5
Total ABO-compatible offspring	2251	2288.6	2462	2201.6																
Total ABO-incompatible offspring	636	598.4	316	576.4																
Offspring of *c* matings	1753	1855.8	2058	1784.9																
Offspring of *i* matings	1134	1031.2	720	993.1																

TABLE V. DISTRIBUTION OF PARENT-OFFSPRING BLOOD GROUP COMBINATIONS

Offspring	Series	Ascertainment	Mothers					Fathers				
			O	A	B	AB	Total	O	A	B	AB	Total
O	Rh NS	Single	876	365	133		1374	654	286	88		1028
		Multiple	1027	432	148		1607	805	348	114		1267
	Rh S	Single	462	183	52		697	383	170	39		592
		Multiple	1024	395	97		1516	880	354	90		1324
A	Rh NS	Single	344	695	49	54	1142	254	544	34	35	867
		Multiple	407	828	57	64	1356	306	695	42	42	1085
	Rh S	Single	74	395	7	23	499	148	261	15	20	444
		Multiple	204	850	20	50	1124	324	629	33	31	1017
B	Rh NS	Single	146	62	152	46	406	76	44	125	50	295
		Multiple	169	69	178	60	476	90	59	162	54	365
	Rh S	Single	21	13	56	20	110	37	14	39	6	96
		Multiple	43	32	152	50	277	94	31	101	15	241
AB	Rh NS	Single		50	46	19	115		45	38	11	94
		Multiple		59	59	20	138		54	46	11	111
	Rh S	Single		13	5	11	29		14	7	5	26
		Multiple		31	25	25	81		29	18	16	63
Total	Rh NS	Single	1366	1172	380	119	3037	984	919	285	96	2284
		Multiple	1603	1388	442	144	3577	1201	1156	364	107	2828
	Rh S	Single	557	604	120	54	1335	568	459	100	31	1158
		Multiple	1271	1308	294	125	2998	1298	1043	242	62	2645

A Differential Method for Estimation of Type Frequencies in Triplets and Quadruplets

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A TWIN GESTATION may result either from release and fertilization of an extra ovum or from division of one zygote or early embryo. It is known that in the higher orders of plural birth these processes are often combined, so that triplets may derive from one, two or three ova, quadruplets from any number up to four. The relative frequencies of the possible zygosity types among triplets, quadruplets and quintuplets have long been a subject of speculation (Jenkins, 1929; MacArthur and Ford, 1937; Jenkins and Gwin, 1940; Newman and Gardner, 1942). These frequencies must be estimated before it is possible to compute the probability that a higher order plural birth represents a particular type involving division of one or more zygotes.

Das (1953, 1955) was apparently the first author to recognize the principle that the probability of zygote division in a single gestation should increase with the number of zygotes or pre-embryos. Allen and Firschein (1957) obtained a relatively simple set of expressions for plural births by assuming that the total probability of subsequent division is the same for undivided zygotes and for each product of division, and that the frequencies of gestations with different numbers of ova form a simple geometric series. This approach was successful in reproducing the observed proportions of sex-concordance types (same-sex *vs.* mixed-sex) among triplets, quadruplets and quintuplets, but appeared to overestimate the total frequencies of plural births except among Japanese. Bulmer (1958a) derived a single expression to represent the frequency of any type of plural birth by assuming that successive divisions represent a Yule or "pure birth" process, extra ovulations a Poisson process. The Yule formula, as applied to monozygotic twinning, differs from that of Allen and Firschein in defining the average rate of multiplication instead of the total probability of multiplication per zygote or embryo. Bulmer's formula not only reproduced the observed proportions of sex-concordance types, but it closely predicted the total frequency of triplets. For quadruplets, however, it underestimated the total frequency.

The differences between the formula of Allen and Firschein and that of Bulmer are actually large, especially in their predicted proportions of the different zygosity types. Their close agreement with the observed proportion of mixed-sex sets shows that such agreement is an inadequate test of the formulas. The reason

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is now apparent. Triplet or quadruplet sets generated purely by zygotic division will be of same sex, while those generated purely by extra ovulation will be predominantly of mixed sex. Both same- and mixed-sex sets are frequent in the gestations where zygotic division and extra ovulation are combined (referred to hereafter as "combined types" of plural births). Hence, any hypothesis under which the combined types predominate, or which keeps the two pure types in balance, will nearly reproduce the rather even division between mixed-sex and same-sex sets seen in known triplet and quadruplet populations.

The high repeat frequency of two-egg twinning (Bulmer, 1958b) casts doubt on the possibility of predicting frequencies of triplets from simple twin-frequency data. Two-egg twin births tend to select mothers who have a higher than average probability of multiple ovulations, and three-egg triplets would select mothers with an even higher probability. Quite clearly a different approach is needed. One such attempt was made by Peller (1946). Another approach is afforded by a mathematical extension of Weinberg's differential method (Weinberg, 1902; cf. Bertillon, 1874), which for twins gives the proportions of one-egg and two-egg types that exactly fit the given twin population both in sex-concordance and in numbers. In the case of twins, such an estimate is made possible by the recognition of a simple algebraic relation between the number of mixed-sex sets and the number of two-egg sets. This leads immediately to an estimate of the number of two-egg sets, and the number of one-egg sets is then obtained by subtraction.

Mixed-sex triplets, like twins, exclude monozygotic sets, but they include combinations of extra ovulation and primary division of one zygote. It seems likely that such combinations depend on the separate probabilities of these two events as estimated in twins. If so, the number of two-egg sets is predictable from twins, and half of them would be of mixed sex. The number of three-egg sets can then be computed from the number of mixed-sex triplets remaining after subtraction of two-egg sets. Finally, the number of one-egg sets of triplets will be those remaining after all two-egg and three-egg sets are subtracted from the total.

A GENERAL FORMULA

It appears that all attempts to formulate the frequencies of plural births have assumed that the probability of division of any single zygote or pre-embryo is independent of what happens to others in the same gestation; this assumption seems to be permissible until contrary evidence arises. It has also been assumed by most authors that the probability of two or more divisions is a simple function of the probability of one division and that an equally simple relation exists between the probabilities of one and more extra ovulations. These last two assumptions, while very convenient, are not necessary. Allen and Firschein used these assumptions in deriving their simplest formula, but they recognized that the probabilities of successive divisions or ovulations might differ. In the present approach, these probabilities are assumed to differ and to require separate estimation. They will be designated with the following symbols:

a, a', a'', etc. Probability of primary division of a zygote and, respectively, of secondary, tertiary or later division of any derived embryo. An alternate interpretation of these parameters is given in the discussion.

b, b', b'', etc. Probability of extra ovulations, first, second, third, etc., among gestations in which at least the next lower number of ovulations occurs.

As used in the general formula, these parameters describe processes of ovulation and early development, not probabilities at birth. The values of a and b, for example, are larger than the relative frequencies of identical and fraternal twins both because of some of these gestations go on to higher multiples and because some are reduced to single births or end in abortions.

Figure 1 illustrates a model of embryo multiplication upon which the present general formula is based. The diagram differs from that of Allen and Firschein chiefly by the vertical arrows. Each figure within a block adds a new step in multiplication, and the term at the left of each such figure is the conditional probability that gestations will take that step, having completed the preceding one. An arrow indicates that the process below increases the number of embryos by two or more in a single step. Thus, monozygotic quadruplets resulting from division of both primary division products are treated in the calculations as if derived from twin embryos rather than from triplet embryos. Their total probability therefore includes the factor a, as well as a'^2, but not the factor for monozygotic triplet embryos, $2a'$. This method of computation, replacing that of Allen and Firschein, follows from elementary combinatorial theory. Two zygotes have three possibilities: (1) to undergo no division, $(1 - a)^2$; (2) to undergo one division, $2a(1 - a)$; or (3) to undergo two divisions, a^2. When binomials of appropriate order are followed in the derivation of all types of plural birth, the

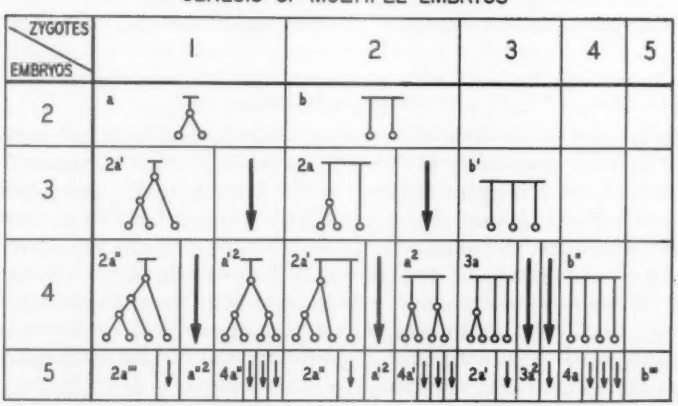

FIG. 1. Steps in the genesis of multiple embryos by binary division of single or multipl zygotes.

complete formula not only satisfies the test of summing to unity, but it can reproduce the predictions of Bulmer's formula (Bulmer, 1958b) after substitution of a special set of values for a, a', b, b', etc., to be discussed in the last section.

The general formula proposed in Table 1 corresponds spatially to Figure 1 except that the rows have been changed to columns, the columns to rows. Also, the table represents births, or gestations at the end of embryo multiplication, and accordingly all terms except those in the last column show a proportion of cases subtracted to allow for further multiplication processes. The last column gives the proportions of quintuplets and all higher births; hence, all terms in the table, like the proportions of all types of births, should add to unity. Examination of the contents of any set of brackets will reveal that they are equal to one. Of the terms or factors outside the brackets, all cancel except the first, which is one.

Table 1 may be directly converted to probabilities for all types of gestation, except that the resulting expressions for quintuplets include all higher numbers of births derived from the same number of zygotes. The probabilities for each type of twins and triplets were given by Allen and Firschein. For quadruplets, they are as follows:

$$\begin{aligned}
&\text{Monozygotic: } a(1-b)[4a'a''(1-a')(1-a'')(1-a''')^2 + a'^2(1-a'')^4] \\
&\text{Dizygotic: } b(1-b')[4aa'(1-a)(1-a')(1-a'')^2 + a^2(1-a')^4] \qquad (1) \\
&\text{Trizygotic: } bb'(1-b'')[3a(1-a)^2(1-a')^2] \\
&\text{Tetrazygotic: } bb'b''(1-b''')(1-a)^4
\end{aligned}$$

SIMPLIFICATION OF THE FORMULA

After factors and terms within gestation types are collected as above, multiplication of any compound term by N, the number of all births, gives the theoretical frequency of gestations with that particular combination of ovulations and divisions. Also, since all factors of the form $(1 - p)$ are very nearly one, they can be neglected in a first approximation. Thus, aN and bN are close to the total numbers of identical and fraternal twin births, although in theory they include the derived higher multiples. Application of the formula may be further simplified by use of symbols for the frequencies of each multiple birth type:

$aN \simeq M$ = Number of monozygotic twin sets in the population studied.
$bN \simeq D$ = Number of dizygotic twin sets in the population studied.
T_s = Number of triplet sets of same sex.
T_m = Number of triplet sets of mixed sex.
T_1, T_2, T_3 = Numbers of triplet sets arising from one, two and three eggs, respectively.
Q_s, Q_m = Numbers of quadruplet sets of same and mixed sex.
Q_1, Q_2, Q_3, Q_4 = Numbers of quadruplet sets arising from one, two, three and four eggs.

When these symbols are substituted into the general formula and all factors

TABLE 1. PROBABILITY OF DIFFERENT TYPES OF BIRTH IF NO FETUSES DIE

Number of Ovulations	Single Births	Twins	Triplets	Quadruplets	Higher Numbers
One	$1-a(1-b)-b$	$+a(1-b)\left\{(1-a')^2\right.$	$+2a'(1-a')\left\{(1-a'')^2\right.$	$+2a''(1-a'')[(1-a''')^2]$	$+1-(1-a''')^2]$
					$+a''^2$
				$+a'^2[(1-a'')^4]$	$+1-(1-a'')^4]$
Two		$+b(1-b')\left\{(1-a)^2\right.$	$+2a(1-a)\left\{(1-a')^2\right.$	$+2a'(1-a')[(1-a'')^2]$	$+1-(1-a'')^2]$
					$+a'^2$
				$+a^2[(1-a')^4]$	$+1-(1-a')^4]$
Three			$+bb'(1-b'')\left\{(1-a)^3\right.$	$+3a(1-a)^2[(1-a')^2]$	$+1-(1-a')^2]$
					$+3a^2(1-a)$
					$+a^3$
Four				$+bb'b''(1-b''')[(1-a)^4]$	$+1-(1-a)^4]$
Five and More					$+bb'b''b'''$

of the form $(1 - p)$ are omitted, the following expressions result for gestations up to quintuplets:

Single births: S

Twins: $\mathrm{M} + \mathrm{D}$

Triplets: $2\mathrm{M}a' + 2\mathrm{D}a + \mathrm{D}b'$

$(\mathrm{T}_1) \quad (\mathrm{T}_2) \quad (\mathrm{T}_3)$ (2)

Quadruplets: $(2\mathrm{T}_1a'' + \mathrm{M}a'^2) + (2\mathrm{T}_2a' + \mathrm{D}a^2) + 3\mathrm{T}_3a + \mathrm{T}_3b''$

$(\mathrm{Q}_1) \quad (\mathrm{Q}_2) \quad (\mathrm{Q}_3) \quad (\mathrm{Q}_4)$

Quintuplets: $(4\mathrm{T}_1a''a''' + \mathrm{T}_1a''^2 + 4\mathrm{M}a'^2a'') + (4\mathrm{T}_2a'a'' + \mathrm{T}_2a'^2 + 4\mathrm{D}a^2a') + (2\mathrm{Q}_3a' + 3\mathrm{T}_3a^2) + 4\mathrm{Q}_4a + \mathrm{Q}_4b'''$

STEPS IN THE ESTIMATION OF TYPE FREQUENCIES

The values of M and D may be estimated from birth data by Weinberg's differential method:

$$\mathrm{D} = 2 \cdot (\text{opposite-sex twins})$$

$$\mathrm{M} = (\text{same-sex twins}) - (\text{opposite-sex twins}).$$

Theoretically, the best estimates of a and b cannot be obtained from twin data without some knowledge of type frequencies in the higher multiple births. Actually, M/S and D/S are very good approximations, considerably better than M/N and D/N. This can be explained from the exact expressions for M and S in Table 1, together with the assumptions that a' is not very different from a, and that both are small:

$$\frac{\mathrm{M}}{\mathrm{S}} = \frac{a(1-b)(1-a')^2}{(1-a)(1-b)} \simeq a(1-a'). \tag{3}$$

Dizygotic triplets result from monozygotic twinning in either partner of a pair of dizygotic twin pre-embryos. The expected frequency may therefore be obtained from the number of dizygotic twins (which is a good estimate of the total of dizygotic gestations) multiplied, since there are two zygotes, by twice the probability of primary embryonic division, a. As in formulas (2):

$$\mathrm{T}_2 = 2\mathrm{D}a.$$

Half of these two-egg triplets are of mixed sex, and after that number is subtracted from all mixed-sex triplets, the remainder represents three-fourths of the three-egg sets:

$$\mathrm{T}_3 = \tfrac{4}{3}(\mathrm{T}_\mathrm{m} - \mathrm{D}a).$$

The number of one-egg triplets is obtained by subtraction:

$$\mathrm{T}_1 = (\text{all triplets}) - \mathrm{T}_2 - \mathrm{T}_3.$$

TABLE 2. FIRST AND SECOND APPROXIMATIONS TO THE PARAMETERS OF EMBRYO MULTIPLICATION FOR U. S. WHITES, 1952–1954

Parameters	a	b	a′	b′	a″	b″
First approximations	.003853	.006403	.002747	.003772	.002217	.01088
Standard errors	.000032	.000035	.000307	.000432		
Second approximations	.003859	.006411	.002745	.003814	.002231	.01085

Approximations for a' and b' can be obtained directly from the estimated numbers of monozygotic and trizygotic triplets:

$$a' = T_1/2M, \qquad b' = T_3/D.$$

For triplets, as for twins, the estimates obtained with these equations exactly fit the data. Within the simplified model, they give a unique solution and are equivalent to maximum likelihood estimates. The exact model, on the other hand, involves higher orders of birth in the estimates for triplets, and full efficiency would require simultaneous estimation of all parameters by a maximum likelihood procedure. However, the information contributed by the highest multiple births is trivial. Close approximations to the efficient estimate can be obtained by use of the first estimates of a′, a″, b′, b″ in the exact formulas, and a comparison of the two sets of estimates in Table 2 shows that the simplified model is as precise as the standard errors warrant.

Simultaneous estimation of sex-ratios and of maternal age effects by maximum likelihood would theoretically bring more information to bear on the estimates of zygosity class frequencies. It has been shown before that consideration of these variables has only a very minor affect on twin estimates by Weinberg's differential method (Strandskov and Edelen, 1946; Allen and Firschein, 1957), and the same is to be expected for triplets and quadruplets.

In analogy with dizygotic triplets, the combined types of quadruplets can be estimated from the probabilities already obtained for divisions in the genesis of twins and triplets. Three-egg quadruplets are simply the fraction of three-egg gestations (approximately T_3) in which one of the three zygotes has undergone monozygotic twinning:

$$Q_3 = 3T_3a.$$

Three-fourths of these will be found among the mixed-sex quadruplets.

Two-egg quadruplets may originate in two ways. Dizygotic twin embryos may both undergo monozygotic twinning, with probability a^2, or either of them may produce monozygotic triplets, with probability $2a \cdot 2a'$. The second process is equivalent to a secondary division, with probability a', occurring in either monozygotic member of a triplet set derived from two eggs. Hence, as in formulas (2),

$$Q_2 = Da^2 + 2T_2a'.$$

One-half of these quadruplets will be of mixed sex.

To estimate the number of tetrazygotic quadruplets, three-fourths and one-

half, respectively, of the above two types are subtracted from the mixed-sex quadruplets, and the remainder is divided by 7/8.

$$Q_4 = \tfrac{8}{7}[Q_m - \tfrac{9}{4}T_3a - \tfrac{1}{2}(Da^2 + 2T_2a')] \quad (4)$$

The monozygotic quadruplets are those remaining:

$$Q_1 = (\text{all quadruplets}) - Q_2 - Q_3 - Q_4 .$$

Estimates of a'' and b'' are given by solution of the expressions for Q_1 and Q_4 in formulas (2).

Unlike twins and triplets, quadruplets yield data with one more frequency observation than the number of constants to be estimated. A maximum likelihood estimate would employ separate frequencies for the two kinds of mixed-sex sets, with sex distributions 3:1 and 2:2, but the improvement would be small. This is shown first by the close agreement of observed frequencies with frequencies calculated in Table 5 on the basis of the simple estimates (see below). Secondly, it is possible to compare the results obtained when each kind of mixed-sex set is excluded, in turn, and these are the extreme possible values under maximum likelihood. This is accomplished in the following two formulas (cf. formula 4), in which $Q_{3:1}$ and $Q_{2:2}$ are the numbers of these two kinds of quadruplets, together equal to Q_m :

$$Q_4 = 2(Q_{3:1} - \tfrac{3}{2}T_3a - T_2a')$$

$$Q_4 = \tfrac{8}{3}(Q_{2:2} - \tfrac{3}{4}T_3a - \tfrac{1}{2}Da^2).$$

Resulting estimates of a'' and b'', to be compared with those in Table 2, are .0023 and .0094 when the 2:2 sets are excluded, and .0021 and .0128 when the 3:1 sets are excluded. These do not appear to differ seriously from each other, and the values given in Table 2, essentially weighted means, are presumably close to the maximum likelihood estimates.

The increased precision of the exact equations, represented in the "second approximations" in Table 2, is no greater for quadruplets than for triplets. It should be noted that, in the case of quadruplets, no direct estimates of $(1 - a''')$ and $(1 - b''')$ are possible; $(1 - a'')$ and $(1 - b'')$ are substituted as preferable to unity. Analogous substitutions would be needed for triplets if useful quadruplet data were not available. In view of the small differences between the first and second approximations in Table 2 (six or more significant figures were used in the computations), the errors introduced by this substitution cannot be large.

The largest difference occurs in the value of b', estimated from triplets. This is perhaps due to the dependence of b' on $(1 - b'')$, since b'' is found to depart from the regular diminution found for the series a, a', a'', and for b and b'. Even for b', however, the difference between the two methods is much smaller than the standard error.

The standard errors were obtained from variance formulas derived by Dr. Seymour Geisser of the Section on Theoretical Statistics and Mathematics,

Biometrics Branch, National Institute of Mental Health. In the formulas below, P_i is the proportion of all confinements resulting in births of type i, where i takes the value 1 for single births, 2 for mixed-sex twins, 3 for same-sex twins, 4 for mixed-sex triplets and 5 for same-sex triplets.

$$V(a) \simeq \frac{(P_3 - P_2)^2 + P_1(P_2 + P_3)}{NP_1^3}$$

$$V(b) \simeq \frac{4P_2(P_2 + P_1)}{NP_1^3}$$

Variances for a' and b', approximated by means of the Taylor series expansion, comprised 15 and 10 terms, respectively, but only the following were found to enter the first three significant figures:

$$V(a') \simeq \frac{P_5}{4N(P_3 - P_2)^2} + \frac{P_4}{36N(P_3 - P_2)^2} + \frac{P_3(3P_5 - P_4)^2}{36N(P_3 - P_2)^4}$$

$$V(b') \simeq \frac{4P_4}{9NP_2^2} + \frac{4P_2}{9N}\left(\frac{P_4}{P_2^2} + \frac{2}{P_1}\right)^2 + \frac{16P_3}{9NP_1^2}$$

The variances of a'' and b'' could be obtained with maximum likelihood calculations and the trend of the standard errors in Table 2 suggests that the sampling errors for quadruplets may be relatively large. However, the estimates of a'' and b'' are based on a greatly expanded sample, as explained in the next section.

The close agreement in Table 2 between the exact and the approximate formulas (second and first approximations) may be due to near algebraic identity of the two methods, but such identity is not apparent. Alternatively, the agreement may be due to the generally close conformity of plural birth frequencies to Hellin's law. Because successive orders of birth form a geometric series, the initial use of single births instead of total births nearly compensates the analogous substitutions at each higher order of birth, as in the estimation of a (formula 3). As a corollary, in any computation using the exact form of one term it would seem best to use exact expressions throughout.

ZYGOSITY TYPES IN AMERICAN TRIPLETS AND QUADRUPLETS

According to Vital Statistics of the United States, published annually by the National Office of Vital Statistics, there were approximately 10,389,800 reported White deliveries in the years 1952–1954. These included 32,923 sets of opposite-sex twins and 72,547 sets of same-sex twins after correction for incomplete reports (see Allen and Firschein). Hence the number of dizygotic sets may be estimated as 65,846; monozygotic sets, as 39,624. In the same period there were 440 reports of mixed-sex triplets and 533 reports of same-sex triplets, again after correction. Substituted into the simplified formulas of the preceding section, these figures yield numbers and proportions of the three zygosity types as shown in the bottom line of Table 3. Comparative data for Negroes appear in Table 4. For Negroes, the estimates of a, b, a' and b' to be compared with the values for Whites in Table 2 are as follows: .00391, .0101, .00271, .00504. These values

TABLE 3. ZYGOSITY TYPES OF U. S. WHITE TRIPLETS, 1952–1954

		One egg	Two egg	Three egg	Total	
Same-sex sets	No.	217	254	62	533	
	%	40.7	47.7	11.6		100
Mixed-sex sets	No.		254	186	440	
	%		57.7	42.3		100
Total	No.	217.2	507.4	248.4	973	
	%	22.3	52.1	25.5		100

TABLE 4. ZYGOSITY TYPES OF U. S. NEGRO TRIPLETS, 1952–1954

		One egg	Two egg	Three egg	Total	
Same-sex sets	No.	34	63.5	20.5	118	
	%	28.8	53.8	17.4		100
Mixed-sex sets	No.		63.5	61.5	125	
	%		50.8	49.2		100
Total	No.	34	127	82	243	
	%	14.0	52.3	33.7		100

TABLE 5. ZYGOSITY TYPES OF U. S. WHITE QUADRUPLETS

EXPECTED RELATIVE NUMBERS PER 100 SETS CALCULATED FROM COMPOSITE DATA

		One egg	Two egg	Three egg	Four egg	Total
Same-sex sets		11.9	17.8	6.8	3.2	39.7
	3:1		13.1	13.5	12.7	39.3
	2:2		4.6	6.8	9.6	21.0
Total		11.9	35.5	27.1	25.5	100.0

extend to triplets the classic observation that racial differences in twinning reflect differences in the frequency of multiple ovulation rather than in the frequency of zygotic division. For Negroes, both a and a' are nearly the same as for Whites, while b and b' are greater in Negroes than in Whites by 60 per cent and 35 per cent, respectively. Any attempt at explanation of this difference should, of course, begin by correcting for differences between Negroes and Whites in average maternal age.

Quadruplet births, even among Whites, were too few in this three-year period to be informative. From the occurrence of 27 quadruplet reports in the period 1947–1954, the expected number among 10,389,800 White births would be 10.6. The best sex-concordance data for quadruplets seem to be those of Nichols (1952), which were based on 114 sets born between 1915 and 1948. From Nichols' figures, the expected distribution of 10.6 quadruplets would be 4.2 same-sex sets, 4.0 sets with a 3:1 sex distribution and 2.4 sets with a 2:2 sex distribution. When the 6.4 mixed-sex sets are used together in formula (4), proportions are found for zygostity classes as in the bottom line of Table 5, approximately in the whole-number ratios, 2:6:5:4. These agree almost too closely with the numbers of each type among 17 reported quadruplets of known zygosity (Allen, in press): 2:6:4:5.

The division of zygosity types into expected sex-concordance classes can be

TABLE 6. ESTIMATES OF ZYGOSITY TYPE PROPORTIONS IN PERCENT: UNCORRECTED, AND CORRECTED UNDER THREE HYPOTHESES OF REPORTING ERROR (see text)

	One egg	Two egg	Three egg	Four egg
Triplets uncorrected	22.3	52.1	25.5	
Hypothesis				
A	20.4	57.9	21.7	
B	22.5	51.5	26.0	
C	22.5	51.7	25.9	
Quadruplets uncorrected	11.9	35.5	27.1	25.5
Hypothesis				
A	11.1	38.0	25.6	25
B	12.3	34.6	26.8	26.3
C	13.1	32.9	26.6	27.4

done according to the fractions given by Allen and Firschein, and by Bulmer, with one exception. According to the formulation arrived at in this paper, the contributions of DZ quadruplets to the 3:1 and 2:2 sex-concordance classes are not in a simple ratio. Half of all two-egg quadruplets should be of same sex, but the other half should be partitioned according to the two terms for dizygotic quadruplets given in formula (2): T_2a' with a 3:1 sex distribution and $\frac{1}{2}Ma^2$ with a 2:2 sex distribution. The proportions of mixed-sex classes predicted in the right column of Table 5, 39.3 per cent for the 3:1 class and 21.0 per cent for the 2:2 class, agree closely with Nichols' observed percentages, 37.7 and 22.8 (the discrepancy of 0.2 in the sum represents accumulated rounding errors).

DISCUSSION

An obvious weakness of the proposed method, not to be circumvented even in maximum likelihood modifications, is that the combined types (two-egg triplets and quadruplets, three-egg quadruplets) are estimated from data on lower orders of plural birth, while the pure types are obtained as the remaining numbers of actually observed triplets or quadruplets. As a result, underreporting of triplets would, for example, reduce the estimates of one-egg and three-egg sets without altering the estimate of two-egg sets. Sampling errors could have the same effect, positively or negatively, although the standard errors already determined show that with populations of good size, statistical fluctuations do not vitiate estimates based on triplets.

In reality, underreporting of triplets would not occur by itself. Some failures to report even single births, as in the case of early abortions, are inevitable. Nonreporting of twins, especially when one fetus died early in gestation, is presumably intermediate in frequency between that in single and that in triplet gestations. Quadruplet gestations may be expected to have the greatest proportion of nonreporting. Insofar as estimates at each level are based on observations at lower levels, this sort of graduated underreporting is expected to lower all estimates and to reduce the discrepancies somewhat.

The extent of this automatic correction can be illustrated. If the magnitude of the shortages were known, corrections could, of course, be made in the computations. Without such knowledge, it is nevertheless instructive to explore the effects of corrections based upon different hypotheses of underreporting. Table 6 presents a comparison of the calculated proportions with the proportions that might be obtained after correction, i.e., with the hypothetical true proportions. The corrections are based on three conceivable distributions of reporting error. All three distributions ignore deficiencies in single birth reports and specify only *relative* shortages in plural births.

Under hypothesis A, all plural births are uniformly underreported by 10 per cent, so that observed frequencies are increased by 10/9 before use of the formulas. With this type and magnitude of reporting error, the true proportions of triplets would differ from the calculated proportions by between 9 and 18 per cent, and the true ratio of MZ to DZ triplets would be about 0.8 of the calculated ratio. The errors for quadruplets are considerably less.

Under hypothesis B, all twins are underreported by 10 per cent; triplets, by 20 per cent; and quadruplets, by 30 per cent. The corrections obtained in this case are very much smaller, amounting to between 1 and 2 per cent in the triplets, between 1 and 4 per cent in the quadruplets.

Under hypothesis C, perhaps the most likely, the assumed errors are not uniform even within birth categories. Values have been selected so that the average deficiencies for twins, triplets and quadruplets are almost identical with those under hypothesis B. However, the reports of twins, relative to single births, account for 85 per cent of MZ gestations and 95 per cent of DZ gestations. In triplets, .75 of MZ sets, .80 of DZ sets and .85 of TZ sets are reported. For quadruplets the figures are .60, .65, .70 and .75. It might be anticipated that this sort of underreporting, greatest in the one-egg categories, would lead to the greatest errors in estimated proportions. Actually, the corrections used here make less difference in the case of triplets than do those of hypothesis B. In quadruplets the corrections range from 1 to 9 per cent and the true ratio of MZ and DZ quadruplets is 1.2 times the calculated ratio. The last error is in the conservative direction for quadruplet zygosity problems where uniovular origin is suspected.

It does not appear likely that reporting errors seriously distort the estimates obtained for zygosity types by the present differential method. This would occur only if plural birth reports were very much less accurate than ordinarily supposed, or if reporting errors selectively reduce certain sex-concordance classes rather than certain zygosity types. The second circumstance would also invalidate Weinberg's differential method for twins.

A more important factor influencing estimates of zygosity is maternal age. In application of the proposed method to individual sets, triplet and quadruplet analyses are needed for narrow maternal age ranges. Twin calculations for the entire population, however, were shown by Allen and Firschein to be only slightly affected by the combining of maternal age groups. If two populations were to be compared, some adjustment for difference in average maternal age would be necessary.

Without reference to physiology, it seems reasonable to assume, as Bulmer did (1958a), that multiplication in the pre-embryonic period should follow Yule's law for population growth by binary fission and that extra ovulation should be a simple Poisson process. However, Yule's formula requires an estimate of the *rate* of multiplication. Empirical data on twinning refer only to total probabilities, and it is not known whether the time interval suitable for embryonic division is constant or, in particular, whether it may not be extended by division of the embryo. In any event, the complexities of ovarian function and of development throw suspicion on any simple formulation of twinning processes. It is interesting therefore that Bulmer's assumptions do find some support in the present analysis.

Plural births may be said to conform to Bulmer's model if, according to the formula given here, a' and a'' prove to be respectively $\frac{1}{2}$ and $\frac{3}{8}$ as large as b.* These decreasing total probabilities in the general formula correspond to constant rates in the Yule and Poisson distributions. Relative values for U. S. Whites, based on Table 2, are as follows:

a	1	b	1
a'	.71	b'	.59
a''	.58	b''	1.69

The probabilities a, a' and a'' fall off less rapidly than predicted, but in the correct direction and proportions. The ratio of b' to b is close to $\frac{1}{2}$, and the large discrepancy in b'' may result from incongruence of the quadruplet data taken from different sources for these calculations. However, Bulmer found that his formula greatly underestimated the number of quadruplets in several populations, and his later observations on repeat frequency of twinning (1958b) lead to the expectation that b' and b'' should exceed the values given by his model. The relative values found here for b, b' and b'' may therefore reflect the true biological trend.

Finally, it should be noted that, although the present formulation combines a, a' and a'' as if they corresponded to successive events, and b, b' and b'' similarly, they are not so conceived. In ordinary monozygotic twinning the probability, a, may represent several different types of division in the population, occurring at several different stages in the pre-embryonic and early embryonic period. Such variation is attested by the different relations of the membranes and umbilical cords in different sets of identical twins. In identical triplets, however, the expression, aa', may usually represent division at two different stages, in which case neither the first nor the second division would have the full range of possibilities found in MZ twins. Bulmer's model, by allotting "unit time" for all divisions to be completed, adheres perfectly to this principle of mutual interference between successive divisions.

The present model formally adheres to a pattern of successive binary divisions

* Succeeding fractions are 31/96 and 3527/11904 in the a-series and $\frac{1}{4}$ and $\frac{1}{5}$ in the b-series. Irregular as the a-series appears, Dr. Seymour Geisser has pointed out to me that the denominators represent the products of successive odd powers of two multiplied by all preceding numerators; the fractions seem to approach $\frac{1}{4}$ as a limit.

with unrestricted time, but in application to triplets and quadruplets, at least, it allows for two other possibilities: (1) One division may sometimes set back development of the daughter products so that secondary division can occur at the same stage or even an earlier stage than did the primary division. (2) Three or more embryonic centers might arise simultaneously instead of two. This kind of division, possibly illustrated in the case of the nine-banded armadillo (Witschi, personal communication; cf. Patterson, 1913), must be seriously considered for man, and warns against the attachment of any biological meaning to the separate parameters, a, a', etc. They may best be regarded as arbitrary measures of the tendency toward multiple division.

In the case of the parameters, b, b', etc., although here again successive events can not be assumed, a simple biostatistical interpretation is appropriate. Each form of b defines a ratio of gestations with more than n ova to gestations with more than $(n - 1)$ ova.

SUMMARY

On the basis of some plausible assumptions, Weinberg's differential method for estimating the proportions of identical and fraternal twins in a large series can be extended to triplets and higher plural births. For triplets, the expected number of two-egg sets in a population of births would seem to be approximately the frequency of two-egg twins multiplied by twice the probability of zygote division (the frequency of one-egg twins). Half of these triplets should be of mixed sex, and subtraction of that number from all mixed-sex sets should leave ¾ of the three-egg sets. A second subtraction yields the number of one-egg triplets. For quadruplets, analogous calculations employ the computed class frequencies of both twins and triplets in a more complicated set of equations. The exact algebraic representation of the zygosity classes for the higher orders of plural birth, derived by combination and expansion of binomials, can be simplified with little loss of accuracy. A number of sources of error are considered and assessed as tolerable for present purposes.

When applied to U. S. birth statistics, the method indicates that the ratio of one-egg, two-egg and three-egg triplets is approximately 1:3:2 among Negroes, and 1:2:1 among Whites. The four ovularity types of White quadruplets occur approximately in the proportions 2:6:5:4.

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BOOK REVIEWS

Genetic Basis of Morphological Variation

By R. H. Osborne and F. V. De George. Harvard Univ. Press, 1959, xxii + 204 pp. $5.00.

The authors have attacked the problem of the genetic study of morphological variation, which is a study of complex multifactorial inheritance, a field which has for the most part been avoided in human genetics. They have done this by studying 207 pairs of twins, 131 of them at first hand. They have attempted to evaluate the twin method in the light of present knowledge of twinning and to apply the method to the detection of complex genetic variability and to the analysis of genetic-environmental interaction.

It would not be possible to summarize here the methods used to acquire the twin sample, to diagnose zygosity, to measure and record morphological features, and to analyze the differences between the twin pairs. The authors give adequate discussions of all these points. Let it suffice to say that they weighed the subjects, measured then anthropometrically, estimated fat, bone and muscle, somatotyped them, and rated them on a masculinity-femininity (this word is hard to spell; Osborne and De George misspell it in large type on p. 146) scale.

To summarize the authors' conclusions is difficult, but some of the high points may be singled out. The analyses of the ponderal index (height/$\sqrt[3]{\text{weight}}$) and of different anthropometric indices clearly show, according to Osborne and De George, that such ratios are gross over-simplifications of the biological relations they purport to describe, and these authors think they are of extremely limited value for genetic studies. The authors believe that the influences that affect the relations between two measurements in a monozygotic twin have a similar affect between the two members of the twin pair. A limited number of anthropometric measurements are thought statistically to measure a genetic component of variability. The genetic component of somatotypology is thought to rest primarily upon the aspect that describes relative linearity.

In summary, it may be said that although this book is by no means the ultimate genetic analysis of human morphological features which both human geneticists and physical anthropologists look forward to eventually, it is a fine beginning, and constitutes the record of a judicious and tenacious attack on this difficult problem.

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Retinitis Pigmentosa Combined with Congenital Deafness; with Vestibulo-Cerebellar Ataxia and Mental Abnormality in a Proportion of Cases

By Bertil Hallgren. Copenhagen: E. Munksgaard, 1959, pp. 101, 19 Tables.

This report on the histories of 177 cases of retinitis pigmentosa with congenital deafness from 72 families represents the first systematic study of a syndrome that was originally

described by von Graefe in 1858. In most instances, the hearing impairment met the criteria of early total deafness and was found to be associated with a vestibulo-cerebellar type of gait disturbance. Intellectual subnormalcy was observed in almost one quarter of the index cases, while the incidence of mental deficiency was not increased in the unaffected sibs.

In the author's opinion, the syndrome is produced by one autosomal, recessive gene with pleiotropic effect, a prevalence rate of 3 per 100,000, and an approximate mutation rate of 2.6×10^{-5}. The parental consanguinity rate (matings between first cousins) was 17 per cent. This figure increased to 34.4 per cent when all consanguineous matings were taken into account. The fertility of affected persons was grossly reduced (0.14 of the general population rate).

Of particular psychiatric interest was the finding of 26 psychotic probands, many of whom had "schizophrenia-like" features, and some of whom had auditory hallucinations (in the presence of total deafness). Although the etiology of psychotic symptoms in these patients was admittedly "difficult to interpret," it was assumed to have been "psychogenic in some cases." To explain the nature of psychogenic influences under the given circumstances, there is a thorough discussion of possible behavioral sequelae of sensory deprivation and unusually stressful life experiences in deaf persons with a progressive visual loss.

The main value of the study may be seen in the careful manner in which clinical symptoms are traced to genetic causes, which in turn are shown to have important practical applications; e.g., the audiometric detection of heterozygotes. Equally important is the fact that in dealing with intricately evolved behavioral correlates, the investigator focuses attention on the inadequacy of narrowly formulated "either-or" theories of etiology. Also, intriguing questions are raised regarding the phenomenology and basic nature of hallucinations.

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A Clinical and Genetico-Statistical Study of Schizophrenia and Low-Grade Mental Deficiency in a Large Swedish Rural Population

By B. HALLGREN AND T. SJÖGREN. Copenhagen: E. Munksgaard, Acta Psychiat. et Neurol. Scand. Suppl. No. 140, 1959, pp. 65.

REPRESENTING the second volume of a comprehensive psychiatric-genetic study of the population of two islands on the west coast of Sweden, this monograph from the Karolinska Institute supplements the previous report on morbidity risks in the general population. (T. Larsson and T. Sjögren. A Methodological, Psychiatric and Statistical Study of a Large Swedish Rural Population. Copenhagen, E. Munksgaard, 1954.) In essence, it contains an analysis of the clinical data on schizophrenia and mental deficiency and offers important statistics on consanguineous marriages and morbidity risks among relatives of affected index cases. The authors continue to avoid the potential pitfalls in studies of this kind, thus making a valuable contribution to the field of psychiatric genetics.

The population studied consists of those persons born and residing in the rural island areas designated A:bo and B:bo. A "period investigation" covering the years 1900–1944 is combined with a cross-sectional investigation as of December 31, 1944. Both the advantages and disadvantages of total population studies are pointed out by the authors.

While the inbreeding factor in the area covered by this study as measured by the prevalence of first-cousin marriages does not appear to differ from that in the general Swedish rural population, the unexpected association found in this population between schizophrenia and mental deficiency can not easily be explained, except on the basis of a high degree of assortative mating.

In addition to a discussion of methodology, the earlier monograph provided evidence for the homogeneity and representativeness of the population investigated as well as estimates of the various morbidity risk figures for the general population, both for 5-year periods from 1901 to 1940, and as of December 31, 1944 as the census date. That report featured a valuable and exhaustive discussion of procedures for the calculation of morbidity risk (expectancy) by the Weinberg and Strömgren methods.

In the present analysis, only the data from the period investigation are used. While the results agree closely with calculations made on the census-day material, the random variations are not as large. The total material consists of 247 schizophrenic index cases, together with 1543 parents and sibs; and of 237 mentally defective index cases, with 1304 parents and sibs. The completeness of ascertainment is indicated by the fact that only one secondary case of schizophrenia and 25 of mental deficiency were found in field investigations; all others had been picked up as index cases through parish registers and hospital records.

Diagnostically, only those cases were accepted as schizophrenic who had typical symptoms and a severe, chronic course. Mental defectives were limited to the low-grade category (IQ less than 50–55).

In the series of 247 *schizophrenics* (115 male and 132 female), the authors present an analysis of clinical sub-groups, clinical symptoms, type of onset and course. The mean age of onset was 28.0 years for males and 32.2 for females, with the majority (54 per cent) having been hospitalized less than two years after having fallen ill, and 38 per cent within one year. There was no correlation of schizophrenia with birth rank in sibships containing affected members, precluding non-genetic influences related to maternal age or position in the family.

The prevalence of first-cousin marriages between parents of schizophrenic index cases was not significantly higher than that in the general population of AB:bo (3.7 per cent compared to 2.6 per cent). However, the authors point out that in a disease which is very frequent in the general population, single-factor recessive inheritance need not be associated with an increased prevalence of first-cousin marriages. In the case of schizophrenia, with a risk in the general population of 1.6 per cent as shown in the earlier report, and with a sib morbidity risk of 7 per cent as revealed here, the frequency of the gene is estimated as over 20 per cent!

The parental schizophrenia rate was no higher than that in the general population, hence lower than that for sibs. The morbidity rate for sibs was higher if at least one parent was psychotic (11.3 per cent compared to 6 per cent); it did not depend to any significant degree upon the sex of the index case.

The morbidity rate for *low-grade mental deficiency* in the general population was 1 per cent for males and 0.75 per cent for females. Among the index cases of this group there was an increased frequency of epilepsy and deaf-mutism.

Consanguineous marriages among the parents of mental defectives were not significantly increased, while morbidity rates were 8.4 per cent for sibs and 2.5 per cent for parents. The latter difference is explained on the basis of reduced fertility.

The most striking finding reported by the authors is a statistically significant association between schizophrenia and mental deficiency, not previously established. To be sure,

this is true only when high-grade deficiency (IQ 55–75) is included, and the authors admit the possibility that the schizophrenic index cases may have been more carefully investigated for the diagnosis of mental deficiency than members of the general population. Considering low-grade mental deficiency only, the morbidity rate among the schizophrenics is not excessive. The same is true for the mental deficiency rate among their sibs as well as for the schizophrenia rate among the sibs of mentally defective index cases. The abnormalities classified as mental deficiency did not appear to be symptomatic of early schizophrenic deterioration any more than did the psychotic symptoms appear to be manifestations of stress in primarily oligophrenic individuals. The series was admittedly not large enough to establish the occurrence of assortative mating as a likely explanation of the observed association, while linkage or penetrance effects represent only hypothetical interpretations at this point. Though the authors clearly state that no definite explanation can be advanced on the basis of the present material, it is observations such as this and the hypotheses they suggest that pave the way for further fruitful research in human genetics.

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An Introduction to Medical Genetics

By J. A. FRASER ROBERTS. New York: Oxford University Press, 1959, 2nd ed. 263 pp., $7.00.

THERE has been a great need for an English language textbook of genetics written for medical students by one well versed in the problems and practices of medical genetics. This need has been admirably fulfilled by Dr. Roberts's book. As the author says in the Preface, "neglect of established principles of medical teaching has been largely responsible for the failure to assimilate and use genetic advances. A textbook of genetics is no more a substitute for one on medical genetics than is a textbook of physiology a substitute for one on medicine". With this in mind the author has assumed that the reader has no previous genetic or special mathematical training, and, by a series of logical and clearly defined deductive steps, has developed from first principles those aspects of genetics that are immediately relevant to the practice of medicine. Illustrative examples, all drawn from the medical field, are liberally supplied.

The concepts of population genetics as they bear on human disease are introduced early, and presented so skillfully that even "those who have an inveterate dislike of algebra of even the simplest kind" will find them illuminating and useful. The final chapter, on "Genetic prognosis" will be very helpful to those who are involved in interpreting genetic principles to members of families in which some problem of hereditary disease has arisen. Dr. Roberts states emphatically that this chapter reflects a personal view, based on his (extensive) personal experience, and his view appears to be a wise one. Some readers will probably disagree with some of his statements, for instance that if, among the relatives of a patient, the chances for normal children are not good, "naturally the subject is not mentioned unless the patient raises it", but this is a question that must be decided by the individual counsellor. The author does not tackle the eugenic aspects of medical genetics, although his statement that "There is no genetic reason why a person with recessive retinitis pigmentosa should not marry and have children" might suggest to the

unwary reader that he is not concerned about the accumulation of deleterious genes in human populations.

Dr. Roberts has chosen not to include a list of human inherited abnormalities which would "necessarily have been incomplete and dogmatic", but has provided a list of key references to sources where such information can be found. This book is intended for medical students, those studying for postgraduate diplomas, and clinicians, and it is heartily recommended, not only to them, but to others who may be involved in teaching genetics to medical students or engaged in genetic counseling.

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Synthesis and Organisation in the Bacterial Cell

By E. F. Gale New York: John Wiley and Sons, 1959, 110 pp.

The author states: "As biochemists throughout the world have laboured on intermediary metabolism and its enzymes, they have produced more and more mechanisms which appear to be the same in Man and Microbe. This has produced a tacit belief that 'fundamental' biochemistry is the same for all living creatures, and the obvious differences between such creatures represent specialisations that protect, buffer, and maintain the fundamental biochemistry against the vicissitudes arising in the course of evolution." The present brief book presents details of the author's own work on protein synthesis in bacteria, in attempting to decipher the intimate biochemical details of this complex process. Although this problem is probably at the level of sophistication that genetics was in 1900, it is clear that two types of reactions are involved in protein synthesis. The first type, called by the author the "individual" type, is concerned with the reactions undergone by single amino acids, while the second, called the "integrating" type, is concerned with the coming together of a large number of amino acid derivatives to produce a specific polypeptide. A few things are now known about the "individual" reactions, but little about the "integrating" reactions. It is hoped that the future will correct this deficiency.

The author writes clearly and concisely, but uncritically. The bulk of the facts presented were obtained in the author's own laboratory, and the interpretations of these facts cannot be accepted without reservation. The large amount of material of other workers is integrated into the text only sporadically, so that this book is a highly personal account. It could not be read intelligibly by non-specialists.

The author is in the Medical Research Council Unit for Chemical Microbiology, Cambridge, England, and this book is based on the third annual Ciba Lectures in Microbial Biochemistry presented at Rutgers University.

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BOOKS RECEIVED

Physiologie und Physiopathologie der Weissen Blutzellen

Edited by H. BRAUNSTEINER. Stuttgart: Georg Thieme, (in the U. S., New York: Intercontinental Book Corporation), 1959, viii + 346 pp. $14.05.

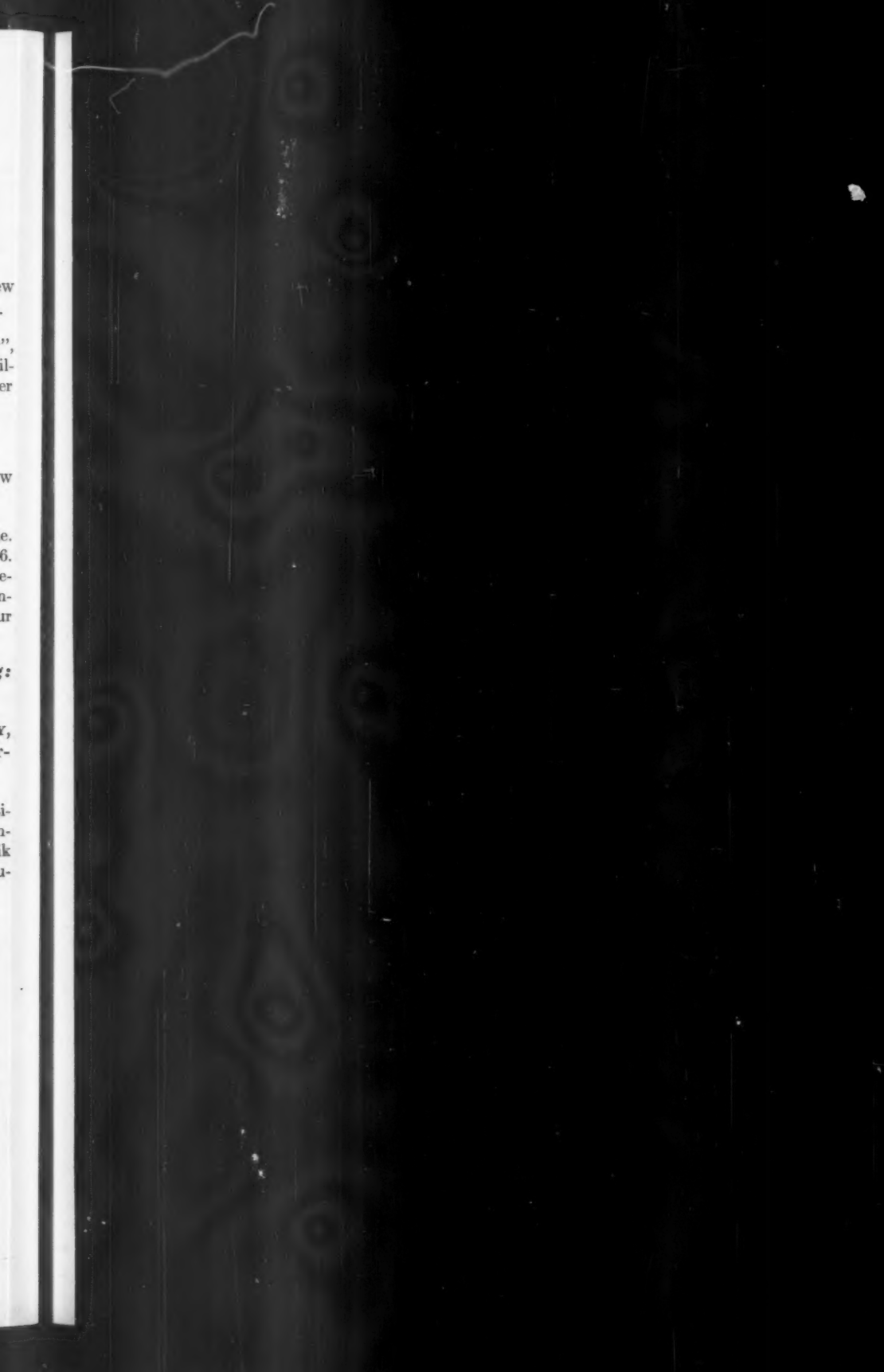
THE sections on "Leukozytäre Antikörper", "Gegen Zellkerne gerichtete Antikörper", "Leukozytenkernanhänge und chromosomale Geschlechtsdiagnose", "Erbliche Missbildungen der Granulozyten", "Homo-und Hetero-transplantation hämatopoetischer Zellen", "Leukämie und Virus" will be of direct interest to the geneticist.

Strahlenbiologie: Grundlagen und Ergebnisse

By HEDI FRITZ-NIGGLI. Stuttgart: Georg Thieme, 1959, (in the U. S., New York: Intercontinental Book Corporation), xvi + 379 pp. dm. 65.

THE twelve chapters of this book are: 1. Physikalische Grundlagen der Strahlenbiologie. 2. Strahlenchemie. 3. Strahlenbiochemie. 4. Strahlengenetik. 5. Zelle und Zellteilung. 6. Die Wirkung der Strahlen auf Embryo, Foetus und sich entwickelnde Lebewesen. 7. Regeneration. 8. Strahlen und Krebs. 9. Strahlenpathologie und Strahlentod. 10. Strahlenkrankheit der Säugetiere und des Menschen. 11. Biologische und chemische Mittle zur Prophylaxe und Therapie der Strahlenschäden. 12. Deutungen, Theorie und Ausblick.

Strahlenbiologie, Strahlentherapie, Nuklearmedizin und Krebsforschung: Ergebnisse 1952–1958

Edited by H. R. SCHINZ, H. HOLTHUSEN, H. LANGENDORFF, B. RAJEWSKY, G. SCHUBERT. Stuttgart: Georg Thieme (in the U. S., New York; Intercontinental Book Corporation), 1959, 998 pp. $65.50.

"DIE Entwicklung der Treffertheorie seit dem Jahre 1946 unter besonderer Berücksichtigung ihrer Anwendung auf die biologische Wirkung energiereicher Strahlen", "Strahlengenetik der Bacteriophagen", "Strahlengenetik der Mikroorganismen", "Strahlengenetik der Drosophila", "Strahlengenetik der Säuger", "Dosierungsprobleme", will be of particular interest to the geneticist.